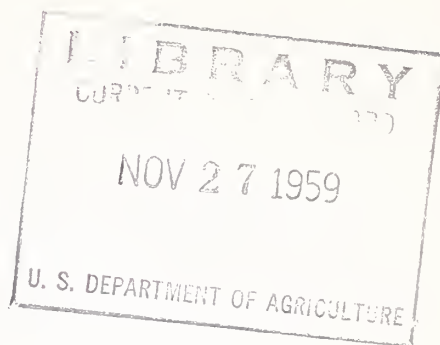


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FIFTH TECHNICAL ALFALFA CONFERENCE

Sponsored by

American Dehydrators Association

and

Western Utilization Research and Development Division
Agricultural Research Service, U.S.D.A.

✓
Colorado Agricultural Experiment Station

Held at

✓ Engineering Hall
Colorado State University
Fort Collins, Colorado

August 15, 1958

PROGRAM

Morning Session

9:40	Industry Observations	Mr. Joseph Chrisman American Dehydrators Assn. Kansas City 6, Missouri
10:10	New Varieties of Alfalfa	Dr. W. R. Kehr Crops Research Div. - ARS University of Nebraska Lincoln, Nebraska
10:50	Gas Storage of Dehydrated Alfalfa	Dr. C. R. Thompson Western Regional Research Laboratory
11:30	Estrogens in Forages	Mr. E. M. Bickoff Western Regional Research Laboratory

Afternoon Session

1:30	Stability of Vitamin E in Alfalfa Meal	Dr. W. E. Pyke Dr. L. W. Charkey Colorado State University Fort Collins, Colorado
2:10	Utilization of Vitamin E in Alfalfa	Dr. G. O. Kohler Western Regional Research Laboratory
2:50	Feeding Dehydrated Alfalfa to Beef Cattle	Dr. John Matsushima University of Nebraska Lincoln, Nebraska
3:30	Feeding Pelleted Alfalfa to Lambs	Dr. L. A. Esplin Colorado State University Fort Collins, Colorado
4:10	Alfalfa for Pigmenting Poultry	Dr. T. M. Ferguson Texas A & M College College Station, Texas
4:50	Dehy - A Source of Roughage Utilization Factors for Ruminant Feeds	Mr. Roy C. Elrod W. J. Small Division Archer-Daniels-Midland Co. Minneapolis 2, Minnesota

SOME INDUSTRY OBSERVATIONS

Joseph Chrisman

American Dehydrators Association
Kansas City, Missouri

The dehydrated alfalfa industry had its beginning in the late 1920's but grew very little until the late 1930's. The major part of the growth from 1930 to 1940 occurred in Kansas; the other plants were springing up in many states from coast to coast.

With the coming of World War II, expansion in this industry, as in many others during the period, was greatly accelerated. Formula feed manufacturing was going into a period of rapid increase in volume and the research departments of those feed manufacturers were diligently exploring all fields for feed ingredients to meet their formula demands. Many of them further implemented their search through grants-in-aid to experiment stations, and the stations themselves became greatly interested in this trend to make commercial utilization of a crop which heretofore had been primarily used only by the farmer who grew it or the stock feeder or dairyman who bought hay or the hog farmer who pastured his hogs on alfalfa, clover or grass fields.

Synthetic vitamins were still, for the most part, relatively high in price. Some of the vitamins had not yet been synthesized and any ingredient which furnished any significant amount of any of the vitamins was of interest. Dehy, as it has come to be known, was an excellent source of carotene (pro vitamin A) and at the same time carried significant and usable amounts of many of the other known vitamins. In addition, it had the glamor of providing an unknown factor or unknown factors of growth-promoting or health-giving propensities.

For these reasons our industry expanded rapidly during and immediately following the War. During the early stages of the War a few of those individuals already in the business of producing Dehy became aware of the need for a united voice in Washington if they were to be able to continue in profitable operation or, in some instances, stay in business at all. A group was organized in Ohio and was chartered in that State as an association to represent the industry of alfalfa dehydration. Other dehydrators in other parts of the country soon realized the value of such an organization in dealing with affairs of common interest to all. Such things as the availability of operating supplies, new machinery, recognition of labor requirements, necessity for adequate price ceilings, transportation requirements, etc., could be presented to much greater advantage through an association than by individuals acting singly, particularly in a war-time economy with its multiple controls.

Following a period of rapid expansion of membership, the headquarters of the association was moved to Chicago, the Ohio charter was cancelled, and

a new Illinois corporation formed. It is under this Illinois charter that we operate today, though the headquarters office was moved to Kansas City in 1951.

When the War was ended it was felt that an end to the acceleration of demand for the product would occur. A few of the dehydrators in anticipation of this possibility began to realize how little we actually knew about our product. Real knowledge of its feeding values and also its shortcomings was confined principally to a few people in the feed business and workers in nutrition research. Selling, instead of being merely a matter of price, dependability and service, now called for a more intimate knowledge of what we had and what it would do for the bird or animal, that something else would not do equally well and perhaps at less cost.

If the industry were to enjoy continued growth it was incumbent upon it to be able to present a case for increased usage, not only where it was currently being used, preponderantly in poultry feeds, but also in feeds for four-footed animals. And so a program of industry supported research was initiated in 1948 and has continued until the present time. It has been implemented through membership dues and a few gifts and has been carried out through grants-in-aid to experiment stations and research foundations throughout the country. You will hear more about this later in the program so I will not dwell upon it. I still consider this program of research at its present status to be inadequate to the needs of the present and future well-being of the industry but without it, I am convinced our industry would not now be enjoying a million-ton distribution of its product and our Association would have withered on the vine.

The occurrence of the Korean War brought renewed vigor to the demand for our product and increased interest in unified action in the industry through the Association. Again we had the whole array of governmental controls which are so necessary when our country goes to war. Again, group action was preferable to individual action, both from the standpoint of government and industry.

Now that we are no longer actively engaged in war, the accelerated expansion of the market has slowed appreciably and the pricing picture has deteriorated substantially. Thanks to the research of recent years as we lost ground in poultry feeds, we picked it up in ruminant and swine feeding; as we suffered lower prices we have offset them, in part at least, by lower conversion costs; as demands for higher quality have been felt, they have been met, in part, by preservative type storage and in a few isolated instances by upgrading in the field and in the plant. I am confident there will be more upgrading in the future.

We will need much help in that phase of the program; help from the agronomist, the soil scientist, the nutritionist, the agricultural engineer, the chemist and biochemist -- and the banker too, no doubt.

Let me talk a little about storage of Dehy under non-oxidizing atmospheres. This is to be covered in a later talk and I will not encroach too far.

It was the hope of the industry and particularly of those who made the substantial investment required for this type of storage, that a leveling out of prices over the 12 months would ensue. This hope was logical from the standpoint that uniform shipments could be made throughout the 12 months of a uniform quality of product; something that could not be done where only atmospheric storage is available except at considerably reduced holding temperatures.

What was perhaps not fully realized was that with adequate stocks of protected product in the winter period the law of supply and demand would work just as well in cold weather as it does in hot. No longer is there much fear of winter stocks inadequate to the demand. Now, just as we always have a glut on the market during the producing season, we also have, not a glut, but at least seldom a shortage of good product in the winter period.

Last winter we witnessed a sad situation where the price began to drop in early December and from that point until well into the new producing season had an almost continuous downgrade. Even with 6 to 9 months' storage charges against it, meal sold just prior to new crop at a price no higher than during mid-July of the producing season. The small excess of stocks over demand was not alone enough to cause such a price drop as occurred. I think it probable that the need for cash to liquidate loans against such stocks ahead of new crop did much to force the market downward.

Many operators of gas storage facilities used them for storage for winter sales only and sold practically nothing during the operating season. Such operators, considering their longer storage period, probably netted no more and perhaps even less than those who stored nothing and sold as they produced. In fact, some of the low-cost, quality-minded producers who sold as they produced, having no burden of high investment in gas storage, were probably in the best position this past year.

There is a wide range in the cost of production among the many plants operating. I have from time to time been privileged to look at cost figures of some plants. I can recall instances of cost-per-ton differentials between plants in the same local area of \$5 or \$6 per ton. When the prices get down around \$30.00 per ton for bulk pellets at the gateway one of those mills is bound to lose money while the other will have a slight profit.

The Department of Agricultural Economics at Kansas State College under a U.S.D.A. grant is now conducting a study of costs of dehydration in the Midwest. Our Association has been cooperating with both agencies, though not advancing any money. When this study is completed and the results published some of the reasons behind such large differences in conversion costs will become more apparent and the high-cost producer may see how he can more ably meet competition.

This sort of research is very much needed as is also other types of research in the forage crop field. We know that dehydration as it is now practiced is not the last word in handling such crops which constitute about half of the total feed of our country. There is the inhibitor of animal growth

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which must be considered. There is the loss of a substantial part of the unknown factors calling for study. We know that we lose a percentage of all the vitamins and growth-promoting factors, though not nearly to the extent that they are lost in field curing. The availability to the animal body of some of the vitamins in Dehy must be more adequately surveyed.

Much interest is presently being shown in the use of forages in compressed forms, both as a supplement and as the forage portion of a complete feed for sheep, beef cattle and dairy cows. More research is needed to determine optimum particle size for best results in dairy cattle feeding or rather, to show whether particle size of itself has any significant effect on milk production, butter fat percentage or total butter fat.

There is a great need for further studies in forage utilization in general. Too great a proportion of the inherent values of our huge forage crops are not being recovered in the production of milk, meat and poultry products.

The type of investigation required for better utilization is such as to make it almost prohibitive for the industry alone to attack the problems. As an instance, fractionation studies are slow, tedious and expensive when conducted on a scale to permit adequate feeding of the various fractions to ruminants or swine. Fractionation just for the purpose of determining total composition and analysis is a very large undertaking. The best we can do as an industry is to provide relatively small sums of money through our Association grants to act as catalysts in furthering much more profound research investigations.

In view of the fact that forage crops constitute about half the total feed of our animal population it is my feeling that some of the many millions of dollars now being spent in crop support might be more profitably expanded on both fundamental and practical research concerned with the production and utilization of forages.

The grasses, using the term broadly, do much to build and to preserve permanent soil fertility but they are running a losing race with the row crops which use it up.

In conclusion, let me say that without the cooperation of the U.S.D.A. through its Agricultural Research Service, Agricultural Marketing Service, the Economics Division and others and without the wonderful help from research scientists, both those who have participated in our grants-in-aid and many who have not and without the cooperation and advice from our own Research Council and the program which it helps administer, we would be so far behind the eight ball we could not even tell it is black.

NEW VARIETIES AND VARIETY DEVELOPMENT OF ALFALFA

W. R. Kehr

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University of Nebraska
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The success of any industry depends first of all on a constant and dependable supply of raw material. As the acreage of a crop plant increases, it seems that problems concerned with its production also increase. Fortunately, research interest also intensifies and research comes to the aid of the producer in many ways. A major aspect of experiment station research on alfalfa has been directed towards reducing the cost of production and assuring a constant supply of the raw product. The role of plant breeding may be looked upon as a cooperative attack to reduce hazards of producing the highest quality raw product obtainable. This cooperation involves teamwork by a group of scientists from many disciplines including genetics, plant pathology, entomology, soils, biochemistry, animal nutrition, and others. The purpose of this presentation is to report completed research as well as research in progress on the development of new varieties of alfalfa with emphasis on the program in Nebraska and the North Central region.

New Varieties and Experimental Combinations

Shortly after the spotted alfalfa aphid was identified and its damage became apparent, research was intensified on the development of varieties which would resist this pest. Fortunately, the variety Lahontan, developed jointly by the U. S. Department of Agriculture and the Nevada Agricultural Experiment Station and released because of its resistance to the stem nematode and bacterial wilt as well as its forage productivity, was found to be resistant. Since this variety appeared to be adapted primarily to areas in the Southwest, and the aphid appeared to be a potential threat to major alfalfa-producing states throughout the country, breeding programs in many states and federal locations were altered to meet this new threat. Methods were developed to rapidly identify individual resistant plants among large populations. While many varieties and populations appeared susceptible as a whole in the seedling and mature plant stages of growth, fortunately, studies of thousands of plants indicated that resistance is rather widespread. This has been particularly true of populations tracing to Turkistan origin.

The variety Moapa was developed jointly by the U. S. Department of Agriculture and the Nevada Agricultural Experiment Station from nine plants selected from the variety African. This is a non-hardy variety expected to give the same performance as African. Certified seed is expected to be available this fall for producers in Nevada, California, and Arizona, where African is adapted (8).

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The Kansas station in cooperation with the U. S. Department of Agriculture furnished seed of an experimental synthetic alfalfa for spring seedings of test plots this year in the Central region. This experimental combination traces to spotted-aphid-resistant plants selected from Buffalo. Synthetics are advanced generations of intercrosses among selected parents.

The Nebraska station in cooperation with the U. S. Department of Agriculture is producing seed of three spotted-aphid-resistant, bacterial-wilt-resistant experimental synthetics this year, Nebraska synthetics 19, 20, and 21. The first of these traces to sources of resistance other than Lahontan and Ranger. The last of these two trace to plants selected from Ranger. The parents of the three synthetics are as highly resistant to the aphid as the most resistant parents of Lahontan.

Several stations have programs incorporating resistance to the aphid into parents previously selected for other traits and subsequently shown to be desirable for producing experimental varieties. Some stations have programs incorporating resistance into experimental varieties found to be adapted locally and in some cases regionally. These programs involve hand-crossing, testing for resistance, back-crossing to adapted parents, and a repetition of these steps several times before the new synthetic is tested for forage production.

In the North Central region, and perhaps in the country as a whole, the importance of the spotted aphid as a constant potential threat to alfalfa production is virtually unknown. Thus, superior-performing experimental alfalfas, whose development predate the entry of this pest into the United States, are potentially new improved varieties. In other words, while it appears relatively easy to incorporate aphid resistance into varieties, this is a time-consuming process. Perhaps 10 years or more are needed before seed of other aphid-resistant varieties will be available commercially. In the meantime considerable benefit may accrue from the release of new varieties with proved performance for traits other than aphid resistance.

At present there are nine experimental wilt-resistant alfalfas which have given good forage yield performance in previous trials in the Central region. These were included in a regional uniform nursery seeded in 12 states in 1956. These 9 have an "A" designation meaning that they were developed through the joint effort of two or more states, including the cooperation of the U. S. Department of Agriculture. By the end of 1958 more definite information on ^{the} performance of these will be known.

Other experimental and recently named alfalfas were included in many of the Central region trials mentioned above. These trials include A600 produced by the Indiana Agricultural Experiment Station (2); Teton, developed and released by the South Dakota Agricultural Experiment Station and recommended by the originating station as a dual-purpose type, i.e., as a hay type from the first cutting and with grass for pasture (1); and Rambler, which has a creeping-rooted habit, drought resistance, winter hardiness, and a level of resistance to bacterial wilt slightly better than that of Ladak (3). Rambler was developed by the Canada Department of Agriculture. The importance of these alfalfas for hay, pasture, and specialized

production such as the dehydration industry demands has not been determined for the region.

Seed of W56, an experimental alfalfa produced by the Wyoming Agricultural Experiment Station, was distributed to several states for regional testing beginning this year (6).

Nutritive Value Studies

The carotene and protein contents of alfalfa must be preserved so that the animal which consumes this feed may profit from this important legume. It is hardly necessary to stress to this group the need for a chemically stable product. In the absence of a usable commercial antioxidant, the Nebraska Agricultural Experiment Station cooperated with the U. S. Department of Agriculture in searching for the possible presence of a naturally occurring antioxidant in commercially available varieties. The varieties Atlantic, Buffalo, Du Puits, Ladak, Narragansett, Ranger, Vernal, and Williamsburg were studied for original carotene content, carotene yield per acre and carotene retention for a two-year period. Samples of these varieties, obtained from a replicated yield trial, were dehydrated from three cuttings per year. A laboratory model dehydrator was used. It was concluded that the varieties were very similar for all attributes studied. In other studies (5) it was found that percent nitrogen and nitrogen yield were similar for two hardy and four non-hardy varieties. This was true for tops as well as for roots.

Utilization of Hybrid Vigor

Another important and fundamental aspect of variety development is research of a basic nature to gain information on how hybrid vigor can be utilized in alfalfa varieties. The Nebraska program, a joint State-Federal cooperative research endeavor, has pioneered in this important aspect. This work which has extended over a period of years under former U. S. Department of Agriculture project leaders stationed at Lincoln, including H. M. Tysdal, B. H. Crandall, and H. O. Graumann, and currently, the writer, is illustrated in the presentation which follows.

At present there is no set pattern for the development of alfalfa varieties. In contrast, most of the corn hybrids in current use are doublecrosses which involve four parents. While a method was published 14 years ago for producing hybrid alfalfa commercially (7), such production has not been realized because parents having the needed traits have not been found. Current emphasis has been on the development and use of synthetics which, as previously defined, consist of advanced generations of intercrosses among selected parents. The principal difference between synthetics and hybrids is that crossing is controlled in hybrid production whereas in the production of synthetics only a percentage of the seed is hybrid, the percentage depending on many factors, because crossing is not controlled except for restricting parentage and by isolation. Examples of synthetic alfalfa varieties are Ranger, Vernal, and Lahontan. While these synthetics have proved to be notable advances, their use has not

permitted capitalization on hybrid vigor to the extent accomplished for example with corn, onions, and cucumbers.

As shown in Table 1, the first generation of synthetics has been decidedly superior to the best check varieties. As generations are advanced, there is a yield decline particularly evident from the first to the second generation. Yields from the second through the fourth generations also decline somewhat but are generally similar. This table represents the average situation. Individual synthetics have not always followed this trend and some have remained stable throughout four generations. The seed planted by the producer may commonly be the fourth generation. Thus in this scheme of seed production in which Breeder, Foundation, Registered, and Certified seed may be the Syn-1, -2, -3, and -4 generations, respectively, hybrid vigor is apparent in the first generation, but is diluted or not found in the generation of seed commonly used by the hay producer. Since maximum yields per acre appear to be obtainable from first-generation synthetics, several methods for commercially producing such seed have been suggested.

One method, termed the Doublecross method, which is comparable in many ways with modern hybrid corn production methods, involves four parents. From the data presented in Table 2, selected from a more comprehensive study, it is seen that forage yields may be stable or may decrease depending on seed generation of the parent used for production. Other data from the study indicated that yields may also rise as the seed generations of the parent are increased. Each synthetic must be studied to determine whether commercial production should be restricted to use of the first generation of the parents or whether an advanced generation of the parents can be used to produce maximum yields.

Another method, termed the Singlecross method, involves the production of Syn-1 generation synthetics from only two parents. As illustrated from preliminary data in Table 3, the yield of two-parent synthetics may cover a wider range than those involving a larger number of parents, with maximum yields obtainable when synthetics are developed from only two parents. This method involves vegetative propagation of parents exclusively. Rooted cuttings are transplanted mechanically in isolated fields resembling a vineyard. The spacing enables cross-cultivation for control of weeds and volunteer seedlings from shattered seed. Seed production per acre from such fields after the first year may approach those obtainable from row seedings since individual plants increase in size as age increases. These fields should persist for long periods since alfalfa is a long-lived perennial. A chief advantage of this method over others is that the seed obtained each year will contain a higher percentage of hybrid seed than obtainable through other methods.

When the above method is extended to include three or more parents it is termed the Polycross method.

With any of these new methods of production greater continuity in the performance of varieties should result than is possible through use of advanced generation seed produced under present certification procedures. In other words, Syn-1 generation seed produced one year should give essentially the same performance as that produced in previous or future years. There is always a potential danger to continuity in performance of a variety when advanced generations are used since each seed increase may introduce problems of accidental mixtures as well as accidental cross-pollination with other varieties. The present certification standards, although based in part on some rather arbitrary decisions, have led to good continuity in variety performance as indicated in Table 4. These data were obtained from a study of 46 lots comprising Foundation, Registered, and Certified seed produced inside and outside of the area of adaptation of the variety Ranger. While a small range existed for all the characteristics measured, no differences were statistically significant.

In contrast to the data on performance of a variety produced under certification rules, data which illustrate the extreme variability in seed lots sold as "Dakota 12" are presented in Table 5. There never was a variety by this name, but much seed represented to be of this identity was sold at one time. Forty-two samples of seed were involved in this study (4). Of these 13 were produced in Nebraska, 21 in South Dakota and the others in various other states. The range observed for each characteristic studied indicates that repeatability in performance would be difficult. These results are typical of those one might obtain from repeatedly seeding Common alfalfa.

With an intensified interest in commercial utilization of hybrid vigor in alfalfa varieties a question arises concerning the possible use of crosses between existing commercial varieties. Preliminary data on some variety crosses are given in Tables 6 and 7. Yield is expressed in comparison with the average of parental varieties in Table 6. A more realistic appraisal of the value of variety crosses is given in Table 7, where yields of variety crosses are compared with the yield of the highest yielding parental variety involved in the cross. It is evident from these preliminary data that the variety crosses studied were inferior in yield to the highest yielding parent and as such did not appear to be of promise for improved yields.

11.

Summary

The variety aspects of alfalfa production with emphasis on completed research as well as research in progress in Nebraska was presented. Variety recommendations are available from each state. Experimental combinations with built-in resistance to economically important pests are in current trials. The varieties commercially available and in current tests were found to be similar in nutritive value. Current emphasis is on the development of synthetic varieties which possess a combination of traits necessary for successful production. At present, maximum yields appear to be obtainable through use of first-generation synthetics.

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Table 1

Forage Yields of 4 Generations of Experimental
Synthetics in Relation to Check Varieties
Lincoln, Nebraska

Synthetic		Generation of synthesis and relative forage yields ^{1/}			
Type	No. Tested	Syn-1	Syn-2	Syn-3	Syn-4
		Percent			
Two-parent	5	108	98	100	100
Multiple-parent	14	107	104	102	101

^{1/} Percent of the average of 6 check varieties.

Table 2

Forage Yields of Synthetics, Depending on Generation
of Parents, in Relation to Check Varieties
Lincoln, Nebraska

Synthetic	Seed generation of parent	Relative forage yields ^{1/} Percent
(C2 x C10) (C17 x C19)	Syn-1 x Syn-1	103
	Syn-2 x Syn-2	104
	Syn-3 x Syn-3	104
(C8 x C21) (C17 x C19)	Syn-1 x Syn-1	112
	Syn-2 x Syn-2	104
	Syn-3 x Syn-3	94

^{1/} Percent of the average of 4 check varieties.

Table 3

Forage Yields of Syn-1 Generation Synthetics
in Relation to Check Varieties
Lincoln, Nebraska

Synthetic		Relative forage yields ^{1/}	
Type	No. Tested	Range Percent	Average Percent
Two-parent	15	93--113	105
Multiple-parent	6	97--108	104

^{1/} Percent of the average of 4 check varieties.

Table 4

Ranger Alfalfa Seed Lot Performance
Lincoln, Nebraska

Characteristic	Range
Field stand	89--100%
Forage yield ^{1/}	92--103%
Spring vigor score	3.8--4.4
Fall vigor score	4.8--5.8
Rate of recovery score	4.8--5.2

^{1/} Percent of the average of 3 check varieties.

Table 5

Variability in the Performance of Seed
Lots Sold as "Dakota 12"
Lincoln, Nebraska

Characteristic	Range
Cold reaction ^{1/}	72--132%
Wilt reaction ^{1/}	18--100%
Spring growth habit score	3.0--5.0
Fall growth habit score	3.0--5.5
Rate of recovery score	4.0--5.5

^{1/} Percent of Ranger.

Table 6

Forage Yields of Variety Crosses in Comparison with
Average Yields of the Parental Varieties
Lincoln, Nebraska

Variety cross	Yield in % of the average of the parental varieties	
	1957	1958
Atlantic x Buffalo	107	98
Atlantic x Narragansett	100	102
Atlantic x Vernal	102	97
Buffalo x Lahontan	104	100
Vernal x Lahontan	97	110
Vernal x Ranger	95	97

Table 7

Forage Yields of Variety Crosses in Comparison with
Yields of the Highest Yielding Parental Variety,
Lincoln, Nebraska

Variety cross	Yield in % of the highest yielding parental variety	
	1957	1958
Atlantic x Buffalo	105	93
Atlantic x Narragansett	95	98
Atlantic x Vernal	98	95
Buffalo x Lahontan	100	90
Vernal x Lahontan	88	98
Vernal x Ranger	93	94

GAS STORAGE OF DEHYDRATED ALFALFA

C. Ray Thompson

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Storage of dehydrated alfalfa in an inert, non-oxidizing gas was begun initially to preserve carotene, pro-vitamin A, but it is now known to preserve vitamin E and probably other labile nutrients. The basis for this process was recognized by studies of several groups. Taylor and Russell (1) at the New Jersey Experiment Station reported in 1938 that carotene in dried alfalfa was preserved when kept in a vacuum or when held at below-zero temperatures. Later, Graham (2) of American Dairies, Incorporated and Quaker Oats Company applied for a patent on an invention which would allow the charging and storing of oxidizable materials in an air tight system and supply a non-oxidizing atmosphere. Wilder and Bethke³ found that dehydrated alfalfa retained its carotene when stored in evacuated cans or under an atmosphere of nitrogen. Hoffman, Lum and Pitman (4) found that storing alfalfa meal at 40° C. in closed containers preserved carotene because oxygen was used up in early stages of storage. They also found carbon dioxide to have little effect.

Professor E. B. Hart (5), University of Wisconsin and co-workers found that sealing alfalfa in heavily waxed cartons or in plastic bags, especially at higher moisture contents (10-12%), preserved carotene. This development was never adopted because of the high cost of bags and leakage caused by normal handling.

The first commercial installation of gas storage equipment was made at Midland, Kansas by Cerophyll Laboratories, Incorporated. This was 2 - 50-ton steel tanks. Later 8 more tanks of equal size were added. Then, 36 - 200-ton tanks were installed. This was as a meal storage plant.

Typical results of storage are given in Table 1.

Since that time the industry has increased gas-storage facilities rapidly as indicated in Table 2.

At present, about 400,000 tons of storage capacity is available.

Investment costs are high but are justified. Tank costs alone are about \$12.00/ton in 500-ton tanks but are lower as capacity is

increased - Table 3. Equipment to supply inert gas also is a sizable item - Table 4. Operating costs are not particularly high but are higher than ordinary warehousing - Table 5.

A small local plant had the following costs for a well-engineered storage plant - Table 6. As indicated, installation costs are about \$18.00/ton.

An interesting approach for storage of dehydrated alfalfa in inert gas is being tried by the Dixon Dryer Company. Four hundred tons of pelleted material is being held under an 80' x 80' polyethylene sheet. The plastic is sealed down with mastic and a light positive gas pressure is held on the system at all times. Billowing of the plastic is a problem. How this storage compares with more conventional means remains to be determined.

Table 1.

Carotene Retention in Atmospheres of
Various Oxygen Content

% Oxygen	Time, Days			
	: 28	: 58	: 85	: 112
	%	%	%	%
0.3	98	97	96	--
3.0	91	93	91	90
5.3	94	92	90	87
Air	76	67	65	61

Table 2

Inert Gas of
Dehydrated Alfalfa Storage in U. S.

	<u>1954</u>	<u>1955</u>	<u>1956</u>	<u>1957</u>	<u>1958</u>
Kansas	44,500	67,500	76,750	97,500	82,300
Colorado	1,610	2,800	10,750	16,500	22,500
Nebraska	72,400	85,320	97,000	106,000	170,000
Ohio	2,400	7,400	8,000	13,300	36,000
Iowa		5,000	5,000	5,000	8,700
Indiana		1,200	2,500	2,600	3,200
Minnesota			3,000	3,500	4,200
Missouri			2,000	3,000	8,500
Oklahoma			600	3,600	3,600
California				6,000	6,700
Illinois				400	2,800
Pensylvania				3,900	3,900
Tennessee					18,000
Georgia					12,000
	<hr/>	<hr/>	<hr/>	<hr/>	<hr/>
	120,910	169,220	205,600	261,300	382,400

Table 3

Storage Tank Costs

		<u>Cost/Ton</u>
Sealed Concrete -	*500 tons each	\$12.80
Steel	- 500 tons	12.00
"	- 4,000 tons	8.00
"	- 6,500 tons	6.00
"	- 10,000 tons	5.00

*20 or more units.

Table 4

Inert Gas Equipment Costs - 11,000 Ton Plant

Water Lines.....	\$ 300
Gas Lines.....	300
Water Disposal.....	100
Gas Distribution Headers.....	250
Gauges, Other Pipe.....	1,260
Pressure Relief Valves.....	840
Installation of Pipe.....	525
Coating for Bins.....	12,600
Gas Generator, Refrigerator Unit, and Dryer.....	12,000
Generator Installation.....	<u>1,000</u>
	\$29,175

Table 5

Direct Plant Operation Costs - 32,000 Tons

	<u>Cost Per Month</u>
Fuel, Power, Water	\$500
Maintenance and Operating Cost	300
Repairs	<u>50</u>
	\$850

$$\$850/32,000 = 2.7¢/\text{ton/month.}$$

Table 6

Gas Storage Costs in Steel Tanks

4-550-ton tanks, 26' diam. x 48'	\$30,000
Conveying system	20,000
Gas generating system	6,000
Two automatic scales	6,000

5,600 cu. ft. natural gas/day - \$2.00

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X
RECENT WORK ON ESTROGENS IN PLANTS

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Last July, at the Fourth Technical Alfalfa Conference, which was held at Albany, California, I reviewed some of the earlier work that had been reported in the field of estrogen-like compounds in plants. In March of this year, I presented a similar report at the conference of Experiment Station Collaborators of the 11 Western states. Since many of you may have attended either one or both of these meetings, I shall try to present only the most recent work in this field, and perhaps indicate the direction in which I feel this work is proceeding at present.

Both detrimental as well as beneficial effects have been attributed to the estrogens in forage. They have been shown to cause vaginal prolapse in ewes and mammary stimulation in wethers and have been incriminated as a cause of poor reproductive performance and even sterility. They have also been suggested to be the factor responsible for increased milk flow in dairy cattle and increased rate of growth of fattening animals. The conflicting reports on the response of beef steers and heifers to stilbestrol when the animals have access to pasture may be explained by variation in the estrogen content of forages.

Since the now classical work of Dr. H. W. Bennetts of Australia who in 1944 demonstrated that serious reproductive difficulties can occur in sheep grazing subterranean clover pastures, a number of similar observations have been made in this country. Within the past year, Professor H. A. Keener of the Dairy Husbandry Department of the University of New Hampshire has informed us that he has been obtaining results over the past several years that dairy cattle fed ladino clover hay sometimes require more services per conception than do comparable animals fed timothy hay. Also within the past year, a report has appeared in the Journal of Animal Science from the Ohio Agricultural Experiment Station, in which studies were made of the reproductive performance of sheep grazed on ladino clover, birdsfoot trefoil, and bluegrass pasture during three grazing, breeding, and lambing seasons. The ewes grazing all season on bluegrass conceived three weeks earlier on the average, than those grazed all season on ladino clover or birdsfoot trefoil pasture. (See Table I.)

Sixty-six percent of the ewes grazed on bluegrass lambed to first service while only 41 percent of the ewes grazed on ladino clover and 31 percent of the ewes grazed on birdsfoot trefoil lambed to first service. Furthermore, the ewes grazed on bluegrass had a significantly earlier date of first detected estrus than those grazed on ladino clover. Estrogenic activity, as measured by the mouse uterine weight techniques, was detected in the ladino clover and birdsfoot trefoil but not in the bluegrass.

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The Department of Animal Husbandry of Oregon State College recently initiated studies on the effects of red clover pasture or hay on reproduction in sheep in response to complaints by some sheepmen in Western Oregon that a longer lambing period and a lower lambing percentage resulted when ewes were pastured on red clover during "flushing" and breeding. (See Table II.)

Some of their preliminary work was reported last month at the meeting of the Western Section of the American Society for Animal Production held at the University of California at Davis. Their work clearly showed that ewes fed on red clover pasture 17 days before and 51 days after breeding had a considerably longer lambing period than ewes fed either alfalfa hay or red clover hay during the same time. The range in days to complete lambing was 12, 19 and 35 days respectively, for ewes in the alfalfa hay, clover hay and clover pasture groups.

In another paper presented by this Oregon group at the same meeting, it was demonstrated that feeding red clover to mice caused alterations in the size of the follicles on the ovaries and they concluded that compounds present in red clover which interfere with fertility do not behave as either true estrogens or true progesterone but that some of both effects are present.

In a report from this same station earlier this year, Drs. Fox, Kaufman, Mason, and Oldfield found that mice fed fresh red clover or red clover hay failed to litter, indicating definitely that there was a substance present in the clover which interfered with reproduction. Some of the results from this study are summarized in Table III.

Dr. A. N. Booth of our Pharmacology Laboratory has confirmed the effects of red clover on preventing reproduction in mice by feeding crude acetone extracts of clover to mice. The extracts were added to the control diet at a level equivalent to 50% of the diet. He has also been successful in inhibiting reproduction with similar extracts of ladino clover.

Although no attempt was made by the Oregon workers to identify the substances responsible for reproductive disturbances, it is known that red clover contains several estrogenic materials including the coumarin-like estrogen which was isolated in our laboratory last year from clover and which we have named coumestrol. This estrogen has since been found to be present in at least 12 different leguminous plants.

In 1955, Drs. Carter, Smart, and Matrone of the North Carolina Agricultural Experiment Station showed that when a compound closely related to those estrogens, which they isolated from soybean oil meal, was fed to mice at a level of 0.2% of the diet, fewer litters were born. In a later paper, they also showed that the same substance fed at a level of 36 to 72 mg. per day to male mice, depressed testes weight and inhibited formation of spermatozoa. Based on their observations, they concluded that the effects of the material on growth and testicular development differed both qualitatively and quantitatively from those of stilbestrol; the synthetic female sex hormone with which you are all familiar.

Within the last month, our Pharmacology Laboratory has found that feeding either coumestrol or stilbestrol to female mice for two weeks completely prevents subsequent littering. We are now in the process of attempting to determine whether interference with reproduction becomes permanent when mice are returned to an estrogen-free diet and whether the reproductive process is blocked in both sexes.

In summary then, it appears clear from the various reports that serious reproductive impairment can occur in breeding animals if permitted to feed on forages containing excessive amounts of natural estrogens. It may well be the case that numerous instances of unexplained breeding difficulties in farm animals could have been attributable to the natural estrogens present in the feed of the animal.

The estrogens naturally present in forages could assume a Dr. Jekyll and Mr. Hyde character in that under certain circumstances their presence may exert a beneficial or deleterious effect on the animal. I have already discussed some of the deleterious effects. A contrasting situation would be the case if it could be proved that they exert an effect on increasing milk production or on increasing the rate of growth of fattening livestock.

Lush, green pasture has long been recognized as having a stimulating effect on milk production. The possibility that plant extrogens are associated with this effect was first suggested by Bartlett and co-workers (Nature, 162:845 (1948)) at the National Institute for Research in Dairying in Reading, England. They pointed out that a progressive decline in non-fatty solids content of milk occurs during the winter months, followed by a substantial and immediate rise when cows are turned out to pasture in the spring. These workers also pointed out that on "going out to grass," cows often show an increase in milk yield, greater than the amount ascribable to the extra nutrients ingested. These workers assayed samples of forage cut from a number of pastures during the period of active growth and found significant estrogenic activity.

More recently, Drs. Bassett and White of the New Zealand Department of Agriculture attempted to establish a possible correlation between lush pastures, high milk production, and estrogenic activity in the forage. Lactation curves of cows, maintained under two different types of grazing management on a typical New Zealand dairy pasture showed that a sharp rise in milk production occurred during the period of lush growth.

A large number of samples of the pasture were taken at weekly intervals through the period of lush spring growth and assayed for estrogenic activity. No positive responses were obtained. This would seem to indicate that there is no correlation between high milk yield and estrogenic content in the pasture. However, in studying the assay procedure for estrogenic activity employed by these workers, it was found that they employed a procedure which although satisfactory for some types of estrogens, would tend to destroy coumestrol. Therefore, if coumestrol were the dominant estrogen in their white clover as we have found to be the case with ladino clover, negative assays would result. It would seem to me that this work might bear repeating, taking care not to destroy the coumestrol or other estrogens that may be present.

Many workers have used stilbestrol or related compounds to induce udder growth and lactation in dairy animals. Recently, the Department of Dairy Husbandry of Kansas Agricultural Experiment Station reported a study to determine the effect on milk production of feeding low levels of stilbestrol to cows. They employed identical twin cows for their study and measured the milk response during complete lactations. Each cow treated with stilbestrol produced more 4% FCM (fat corrected milk) than did the controls. Furthermore, the treated cows were approximately 6% more efficient in milk production and gained less weight during the test period than did the controls. It would be very interesting to repeat these experiments with estrogenically potent extracts of forages prepared during the period of lush spring growth. If positive results were obtained with these estrogenic plant extracts, then the isolated crystalline plant estrogens should also be evaluated in the same way.

Burroughs and co-workers at Iowa State College reported as early as 1954 and 1955 that feeding 5 to 10 milligrams of stilbestrol, per animal daily, resulted in an increase of about 20% in the rate of gain, reduced the feed requirements, and increased daily feed consumption. Since then, there has developed a tremendous interest in the use of the synthetic hormones to stimulate rate and efficiency of gain in animals and the practice has become widely accepted. It would therefore be of great theoretical interest and perhaps of economic importance to ascertain whether the plant estrogens might also be capable of accelerating the rate of growth of animals. Several recent reports do present indirect evidence in this regard and one report presents some direct evidence that the plant estrogen, genistein, does promote rate of growth in sheep.

At the Iowa Experiment Station, in two separate lamb-feeding experiments, researchers were unable to confirm the growth-stimulating effect of stilbestrol. At the end of the feeding period, there were no noticeable differences between the controls and the stilbestrol-treated lambs. Since it was thought possible that the clover hay on which the lambs were feeding might have been contributing estrogen, assays of the clover were carried out. The estrogen content of the hay appeared to be sufficiently high to elicit an estrogenic response if we can assume that the clover estrogen acts similarly to stilbestrol.

In subsequent work, the Iowa group prepared a crude estrogenic extract from clover hay and added it to the ration of fattening lambs. Results of this study were reported in the December, 1957, issue of the Proceedings of the Iowa Academy of Science. The data presented in this report would seem to support the idea that low-level estrogenic activity found in legume forages contributes to the good feeding qualities of these forages and improved carcass grades. The authors were not able to state whether the effects of these low-level estrogen feeding rates were upon the physiological processes of the animal or upon rumen metabolism.

The individually fed lambs were fed a mixed ration and allowed to feed from an individual self-feeder, 3 hours at each of two daily feedings. The starting ration is listed in Table IV.

The estrogenic activity of clover hay was predetermined by mouse uterine weight assay and the extract added to the molasses at a level of 2 to 3 micrograms of equivalent stilbestrol activity per pound of ration. Stilbestrol treatments were also included for comparison.

Addition of 2 micrograms of stilbestrol per pound of ration or the 15 mg. stilbestrol implant increased rates of gain over the control by 40%. The clover extract promoted rate of gain similar to that of extracted clover hay which was approximately 10% above that of the control. Since a response was obtained with the extracted hay as well as with the hay extract, it does not appear that all of the estrogenic or other growth factors were removed from the clover. Perhaps if a more complete extraction of the hay had been made and the plant estrogens fed at a higher equivalent level, a more pronounced effect might have been obtained. It may be noted that the basal ration contains both alfalfa hay and soybean oil meal, both of which usually contain estrogenic materials. The influence these had on the rate of gain was not measured. Nevertheless, this work is of considerable importance in demonstrating that crude estrogenic extracts of plants can stimulate increased growth response in farm animals. In order to substantiate this fact further, the researchers also performed similar experiments with purified plant estrogen, genistin, and again obtained measurably increased growth rates. (See Table VI.)

The 200 milligram genistin addition per pound of ration was equivalent in estrogenic activity to 4 micrograms of stilbestrol per pound. The 1.5 microgram stilbestrol addition resulted in a 26% increase in rate of gain. The higher levels appeared to be less effective for increasing rate of gain. The genistin addition caused a 15% increase in rate of gain. On the basis of estrogen activity and response to stilbestrol in this experiment, this approximate response might have been expected from addition of 4 micrograms of stilbestrol per pound of ration.

Dr. Matsushima of the University of Nebraska Experiment Station has recently reported some very interesting work in which he also has found that when dehydrated alfalfa is fed as a protein supplement in a cattle-fattening ration, there is less response in gains due to stilbestrol, again suggesting that the potential estrogenic activity in dehydrated alfalfa acts similarly to stilbestrol. Since Dr. Matsushima is attending this conference and will report more completely on this work during this afternoon's session, I shall simply state at this time that his observations may be of great significance to the alfalfa-dehydrating industry, if it can be established that the increased rates of gain obtained with alfalfa meal are indeed due to the presence of plant estrogens acting in the same manner as stilbestrol. Studies conducted by the Iowa Station with crude estrogenic extracts on sheep suggest an approach that could well be profitably extended to studies with cattle.

In connection with such studies, a great deal more information is necessary on the normal variations that can be expected to occur in the alfalfa plant, with different varieties, different stages of growth, season of year, number of cuttings, climatic conditions, and losses during processing. Dr.

Andrews and his co-workers at Purdue University have worked on this phase of the problem for a number of years and have contributed more to our understanding of some of the variables involved than have any other group in the world. In the Report on Estrogens presented at the Fourth Technical Alfalfa Conference, I reviewed some of the work, in which it was shown that estrogen content varies tremendously during the growing season with number of cuttings and stage of development of the plant. An additional report by Drs. Stob and Andrews has appeared in November, 1957, in which 56 different strains of alfalfa were studied. The samples analyzed were second cuttings from individual clones harvested in the bud stage at the clonal nursery at Purdue. Varieties of alfalfa studied included Ranger, Grimm, Hardigan, Cossock, Buffalo, Viking, and others. As expected, a wide variation in estrogenic activity of various samples was found. The increase in uterine weight of immature mice used as assay animals resulting from feeding alfalfa extracts ranged from 1.6 to 82.96 mg., as shown in Table VII.

Differences in the degree of estrogenic activity occurred in all varieties studied, indicating a lack of variety specificity in terms of estrogen content. Dr. Stob informed me in a personal communication received several weeks ago that they have since made rooted cuttings from the clones of alfalfa reported above and with these have established new plantings. They have harvested material for assay purposes from these new plantings within the last month. It will be very interesting to follow their subsequent work with these new plants made from the cuttings to see if the large estrogenic differences found in the original alfalfa carry through the cuttings into the new plants.

At the Western Regional Laboratory, we have also been studying estrogen variability as influenced by varietal and clonal differences and during different stages of growth of the plant. Our studies have been concerned mainly with ladino clover and have been carried out in close collaboration with the Clover Investigations Section, Forage and Range Research Branch, ARS, USDA, which is under the direction of Dr. E. A. Hollowell. Dr. E. G. Beinhart, Jr., who is stationed at Clemson College, South Carolina, has grown a number of clones of ladino clover under both greenhouse and field conditions. These have been harvested at various stages of growth and shipped to our laboratory for bioassay. Similarly to the work reported above with alfalfa, wide differences in estrogen contents were noted among the various clones. Furthermore, the relative estrogenic potencies of the various clones studied did not remain the same from season to season, indicating the complexity of the problem and the number of still unknown variables which will have to be evaluated.

At the Fourth Alfalfa Conference, I reported on Dr. Andrews' findings that alfalfa silage contained appreciably more estrogen than did the comparable alfalfa in pasture. The results appeared to indicate that the ensiling process caused the formation of estrogen-like materials. A subsequent report on the factors affecting the estrogenic content of alfalfa silage appeared in March of this year from the Purdue Station. The intended object of this

study was to determine the effect of stage of maturity, type of preservative, and length of fermentation on the estrogenicity of alfalfa silage. However, the results indicated that type of preservation or ensiling in itself was without effect and concluded that the estrogenic activity of alfalfa silage is more likely to be a function of original activity of the alfalfa than a result of the ensiling process.

Summary

In summary then, it appears clear from the various reports that have been cited above, that serious reproductive impairment can occur in breeding animals if permitted to feed on forages containing excessive amounts of natural estrogens. It may well be the case that numerous instances of unexplained breeding difficulties in farm animals could have been attributable to the natural estrogens present in the feed of the animal.

Furthermore, data appear to be accumulating which suggest that low-level feeding of plant estrogens, normally present in forages, may accelerate rate of gain of fattening animals and improve carcass grades.

Table I

INFLUENCE OF PASTURE ON REPRODUCTION EFFICIENCY

<u>Kind of Pasture</u>	<u>Ewes Lambing to First Service</u>
	Percent
Bluegrass	66
Ladino clover	41
Birdsfoot trefoil	31

Table II

INFLUENCE OF FEED ON LAMBING EFFICIENCY

<u>Treatment</u>	<u>Lambing Percentage</u>	<u>Range in Days to Complete Lambing</u>
Alfalfa hay	156	12
Red clover hay	110	19
Red clover pasture	110	35

Table III

EFFECT OF RED CLOVER ON LITTERING IN MICE

<u>Female Treatment</u>	<u>No. of Females That Littered</u>	<u>No. of Females Not Littering</u>
Control	27	3
Red clover hay*	0	30
Fresh red clover*	0	30

*The clovers were fed as 40% of the dry weight of the diet.

Table IV
BASAL RATIONS FOR INDIVIDUALLY FED LAMBS

<u>Ingredient</u>	<u>Percent of Ration</u>
Ground alfalfa hay	50
Molasses	10
Cracked corn	38
Soybean oil meal	2

Table V
WEIGHT GAINS OF LAMBS FED LOW LEVELS
OF STILBESTROL AND PLANT ESTROGENS

	<u>Addition Per Pound of Basal Ration</u>				
	<u>0</u>	<u>2 mcg. DES./lb.</u>	<u>Hay Extract</u>	<u>Extracted Hay</u>	<u>15 mg. DES Implant</u>
Avg. daily gains, lb.	.34	.48	.37	.39	.49
Avg. daily feed, lb.	2.72	3.20	2.92	3.13	3.00
Grade	5.75	6.00	6.75	5.75	5.00

Animals individually fed for 63 days.

Table VI

WEIGHT GAINS OF LAMBS FED LOW LEVELS OF
STILBESTROL AND GENISTIN

	<u>Addition per Pound of Basal Ration</u>				
	<u>Micrograms stilbestrol</u>				<u>Milligrams genistin</u>
	0	1.5	3.0	6.0	200
Avg. daily wt. gains (lbs.)	.45	.58	.53	.49	.53

Animals individually fed for 42 days.

Table VII

UTERINE WEIGHT RESPONSE TO DIFFERENT STRAINS OF ALFALFA

<u>Alfalfa strain Number</u>	<u>Mean uterine weight</u>	<u>Difference in uterine wt. from control</u>
	mg.	mg.
Control	17.00	--
262	18.60	1.6
339	25.08	8.08
216	30.08	13.08
80	35.80	18.80
346	41.68	24.68
138	99.96	82.96

X STABILITY OF VITAMIN E IN ALFALFA MEAL X

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ABSTRACT

1. A combined method of analysis for the estimation of carotene and tocopherol in alfalfa meal has been developed.
2. A series of alfalfa meal samples with widely scattered origin have been evaluated for carotene, tocopherol and Vitamin K.
3. In the better quality meal samples from the selected areas or from the open market, the initial numerical values for carotene in milligrams per hundred grams dry meal and for tocopherol in milligrams per hundred grams dry meal and Vitamin K in micrograms per hundred grams dry meal did not vary widely and ranged between 20 and 30. In other words, if the carotene value was 23, the tocopherol and Vitamin K values were also about 23 with the dimensions as indicated.
4. Aliquots obtained from the alfalfa meal samples collected were used to study the storage losses of carotene, tocopherol and Vitamin K, at a temperature of 25° C. The aliquots for the storage study were divided in half. One half received no pre-storage treatment, the other half was treated with an antioxidant. These treated and untreated samples were held in storage with the temperature held at 25° C. for twelve-week and for twenty-four week periods. Each aliquot was analyzed for carotene, tocopherol and Vitamin K at the end of its assigned storage term.
5. Losses of carotene during 25° C. storage without antioxidant were more rapid than losses of either tocopherol or Vitamin K. The loss of tocopherol was about 6/10 as rapid as the loss of carotene. The loss of Vitamin K was comparatively very slow under these conditions. Treatment of samples with antioxidant followed by storage under these conditions cut the storage losses in a highly significant manner. The manner of application of the antioxidant greatly influences its effectiveness in arresting losses.
6. The first cutting dehydrated alfalfa meal was superior in the measures made in one-half of the source areas represented. The third cutting alfalfa meal was superior in the other half of the areas.

7. In general, when either the loss of carotene or the loss of tocopherol is considered with a large number of random samples, behavior follows the first order reaction closely. This means that the deterioration rate is limited by one single step in the series of reactions involved. Our data would indicate that when different conditions may be imposed on different sets of similar samples that the limiting step may be changed to another one of quite different character.
8. An equation has been proposed which relates the carotene content of dehydrated alfalfa meal to the tocopherol content. This equation is $Y = 8.5 + 0.6X$, where Y = tocopherol content in milligrams per 100 grams dry meal and X = carotene content in milligrams per 100 grams dry meal. This equation is useful in the usual range of between 10 and 30 for tocopherol content. Its reliability is probably greater between 14 and 34 for tocopherol content. This last statement follows because of the questionable nature of many very low carotene evaluations.

UTILIZATION OF VITAMIN E IN ALFALFA

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I believe that one of the problems in selling dehydrated alfalfa meal is that it is still confused with hay in the minds of many. Alfalfa meal can be sold as a roughage feed and this is a large but low price market. The other market for dehydrated alfalfa meal is as a vitamin concentrate. Alfalfa meal moving into this market must be of as high a quality level as it is possible to produce since this higher price concentrate market attracts competitors like honey attracts flies. For this market, alfalfa should be segregated for quality and upgraded mechanically and chemically. I think it would help if a new name would be developed on an industry-wide basis for the concentrate-type of forage meal to try to disassociate it from hay and roughage. Perhaps Adameal, or Aconomeal, or Adacon would be suitable.

What necessary ration elements are found in alfalfa in high enough quantities to permit rating it as a "concentrate"? There are five such elements for poultry. They are carotene, xanthophyll, vitamin E, vitamin K, and unidentified growth and reproduction factors. It is important to note that of these five, four are unstable. Inert gas storage developed by Cerophyl Labs several years ago partially solves this stability problem for carotene, E and xanthophyll. Stabilization with an antioxidant is necessary to complete this job but Santoquin, the best antioxidant we have found, is still tied up in the F.D.A. Complete retention of unidentified growth factors will require a drastic revision in process and is still in the future.

Each of the positive factors of alfalfa meal is under fire from competitors. My subject today is the vitamin E of alfalfa which has taken a worse drubbing than the others in recent years.

Several years ago at the Third Technical Alfalfa Conference I reviewed for you the status of vitamin E in chick nutrition. I pointed out that field encephalomalacia or "crazy chick disease" appeared to be due partially to a vitamin E deficiency but that certain antioxidants such as DPPD, BHT, and Santoquin had the same effect as vitamin E in preventing the condition. Neither Santoquin nor DPPD is permitted by the F.D.A. BHT is being used in many poultry rations but does not appear to be as effective as the others, so there is still great interest in assuring an adequate supply of vitamin E in the rations of growing chickens and turkeys. In addition it has been shown that chicken, turkey and duck breeder rations require relatively high levels of vitamin E in order to insure fertility and hatchability of the eggs.

As the result, a product has been developed for the feed industry which contains d- α -tocopheryl acetate incorporated in a base of soybean oilmeal. This product is a competitor of alfalfa meal and is sold with claims that its vitamin E is more stable and more "available" biologically than the

tocopheryl of alfalfa meal. It is further claimed that alfalfa actually has been greatly overrated insofar as the actual levels of vitamin E present because of its content of materials which interfere in the chemical determination. We appreciated several years ago that if all of these points were correct, alfalfa meal would lose a selling point which would be of increasing importance. We therefore collaborated with the Association to attempt to develop the facts concerning vitamin E in alfalfa. Results have been slow in coming since funds available have been minimal.

The first part of my talk will concern the amounts of vitamin E in alfalfa meals and the relation of these amounts to the requirements of poultry for vitamin E. I will then discuss work done on the availability of alfalfa vitamin E. Finally I will point out some possibilities for research to resolve the discrepancies which still remain between different research organizations in this field.

The first point which must be considered in discussing vitamin E potency is the difference in activity between the different forms of vitamin E. Let us consider the chemical name of the most important and most potent. It is d. α -tocopherol. There are many related tocopherols, seven of which occur naturally in various feeds and foods. However, in alfalfa at least 95% of the total tocopherol is alpha (F. Brown, 1953). Hence we shall restrict our discussion to the alpha form. Another feature of the name is the "d". Alpha-tocopherol as it occurs naturally is in the "d" (optically active) form. Alpha-tocopherol, as it is synthesized in the laboratory, is optically inactive or is in the d.l. form. Either the d. or the d.l. forms may be obtained as the acetates or other esters, which are more resistant to oxidation than the free alcohols. Some years ago the purest, most stable form available was d.l. α -tocopheryl-acetate. This was therefore usable as a standard so an international committee designated an international unit as 1 mg. of this material. The potencies of all the different forms of tocopherol may be expressed as I.U. on the following basis:

1 mg. d.l. α -tocopheryl-acetate = 1 I.U.

1 mg. d. α -tocopheryl-acetate = 1.36 I.U.

1 mg. d. α -tocopherol (as in alfalfa) = 1.49 I.U.

I should now like to discuss briefly the amount of vitamin E in alfalfa. Dr. Charkey has just presented to you the Colorado results obtained under contract from the Western Regional Laboratory. I have included some of his results together with the results of others in the handout material I have passed to you (Table 1). You will note that the results for the most part range from 100 to 150 I.U./lb. Checks between Harris's and Derse's laboratories are reasonably good. Singsen and Bunnell of the Connecticut Agricultural Experiment Station have obtained somewhat higher results on the same sample, although not enough higher to explain the low availability figures they have reported. The Colorado results (not shown) fall reasonably close to the Connecticut results. The most specific method inherently is that of Fred Brown shown on the first line of Table 1. He actually separated the various forms of tocopherol in feedstuffs and determined them separately. The nature of his samples was not reported but they must have been of extremely high quality since the data of Pyke and Charkey as shown on lines 7, 8, and 9 show that the samples with 150,000 I.U. of carotene per pound contain 116 to 159 I.U. of vitamin E per pound.

On the other handout sheet (Table 2) are some of the estimates in the literature concerning the vitamin E requirements of poultry. The great differences noted are probably due to the degree of "stress" involved if this term is used loosely. Thus in the absence of cod liver oil in the diet it is extremely difficult to produce "crazy chick disease" in young chicks. When .5 to 3% of high quality cod liver oil or other fish oils are added to the ration high levels of E or antioxidant are necessary. In the case of breeder rations the requirements appears to be high even in the absence of highly unsaturated fats.

At the present time I believe the industry considers M. L. Scott's "requirements" to be reasonably close to reality although two factors should tend to reduce the levels. First, the use of antioxidants in feeds is already widespread, and--if and when Santoquin can be used--the requirements will have to be re-evaluated. Second, Scott and Nelson (1955) found that feeding grass juice concentrate reduced the vitamin E requirement. More work is necessary to confirm and extend this observation.

Table 3 shows where the vitamin E comes from in a typical broiler ration. It will be noted that if the alfalfa meal used is high quality--e.g., leaf meal--and if its E is completely available, such a ration almost meets the requirements of chickens but falls far short for growing turkeys. Table 4 shows the typical breeder ration containing 7% alfalfa leaf meal. With hens the requirement is met, but again with turkey hens added vitamin E will be required. This points up the necessity for further work on the requirements of poultry for vitamin E where antioxidants are used in the rations and when adequate sources of unidentified growth factors are fed.

The above calculations are based on the vitamin E of alfalfa meal being correct as determined and being 100% available to the animal. The idea that this might not be true was developed by workers at the Connecticut Agricultural Experiment Station.

Next is shown some of the work indicating this. In Table 5 are some of Dr. Singen's data which show that unit for unit, alfalfa meal is not as effective as d- α -tocopheryl acetate in preventing encephalomalacia in chicks. When Dr. Singen presented this work at the Feed Manufacturers Research Council meeting several years ago, the objection was raised that he was feeding cod liver oil to induce encephalomalacia and that this would destroy some of the alfalfa vitamin E but would not affect the tocopheryl acetate. This was a valid criticism. I went back to Kansas City and got the ADA to collaborate with Dr. H. M. Scott of Illinois and the Wisconsin Alumni Research Foundation to set up experiments to check availability. I also persuaded Dr. M. L. Scott of Cornell to start some work using his special vitamin E low ration. Dr. Singen also went home to do more work omitting cod liver oil from his ration. In Table 6 you will see some of his results using the liver storage method of evaluating availability. He was completely successful in confirming his earlier conclusion that alfalfa tocopherol is only about 25 to 33% available. At every level alfalfa permitted less storage than even dl- α -tocopherol. That the difference was not due to instability is shown by the equal activity of the pure free alcohol form and the pure acetate form. A similar experiment was run by Dr. R. H. Bunnell (1957). His results are shown in Table 7. He agreed that alfalfa tocopherol is very poorly utilized.

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Still another piece of work has appeared which lands a blow on alfalfa's bloody head. Dr. Couch at Texas found that alfalfa meal at a 5% level did not effectively raise the vitamin E content of egg yolks while α -tocopheryl acetate did. If we had not further evidence, we would have to concede that alfalfa was pinned to the mat, that its contribution of vitamin E was not much in proportion to poultry requirements.

But if this were true, it is hard to see how poultry would have gotten along all of these years.

Let me turn now to the results obtained at the Wisconsin Alumni Research Foundation. A preliminary experiment was run in 1955 using the Connecticut basal ration with cod liver oil. In this experiment (Table 7) the ration was stored in the refrigerator. No deficiency symptoms developed and liver levels on the basal ration were high. Little effect on liver levels of E were obtained. Hence, even without alfalfa meal, the ration contained enough vitamin E or the chicks obtained enough storage E from their dams.

In the following year a cooperative experiment was set up by the ADA. Part of the experimental work was done at the Wisconsin Alumni Research Foundation where E availability was tested using two different rations, the Connecticut ration with cod liver oil stored at room temperature and the Cornell cod liver oil-free ration. Another phase of the work was done at the University of Illinois where a third, synthetic type E-low ration was used. All chicks were supplied by the University of Illinois. Three samples of alfalfa meal were analyzed by four different laboratories, the Wisconsin Alumni Research Foundation, Distillation Products, Incorporated, Connecticut Agricultural Experiment Station, and Colorado State University. The results varied somewhat but were in the same range (see Table 1). Sample A was selected and used in the bioassays at Illinois and the Wisconsin Alumni Research Foundation. At the same time a portion of the same sample was sent to Dr. M. L. Scott of Cornell who had agreed to do some comparable tests. The results of Phase I done at the Wisconsin Alumni Research Foundation are shown in Table 9. Here the Connecticut ration was used. The results showed that based on either growth stimulation or liver storage, the vitamin E in alfalfa was utilized as well as d- α -tocopheryl acetate. The results of Phase 2 (Wisconsin Alumni Research Foundation) are shown in Table 10. Here the Cornell E-low ration was used. Growth was poorer and E storage was higher on this ration but again comparison of Alfalfa E and d- α -tocopheryl acetate showed that alfalfa E was at least as effectively or perhaps was even better utilized than the pure acetate form.

Table 11 shows the results of Phase III of the experiment done at the University of Illinois using the synthetic type ration. Unfortunately the livers could not be run since the labels were lost during shipment to the Wisconsin Alumni Research Foundation. However, the growth results shown indicate that E of alfalfa was available. No symptoms of encephalomalacia or edema were found.

Tables 12 and 13 show the results of M. L. Scott at Cornell. He used the Torula yeast diet used in Phase II of the ADA work. The condition which develops on this ration is called exudative diathesis, a fancy term meaning water in the tissues. Here both on a growth and symptom basis alfalfa tocopherol is equivalent to d- α -tocopheryl acetate.

This was very confusing since the difference between these results of Cornell, Illinois, and the Wisconsin Alumni Research Foundation on one hand and Connecticut and Texas on the other can be only partially explained by differences in analytical procedure. I presented these Illinois and Wisconsin Alumni Research Foundation results at the Informal Poultry Nutrition Conference two years ago. M. L. Scott gave a paper on his results at the Federation meetings the same year. It was suggested that we publish the work in a scientific journal but I did not wish to do this since all of the results had been obtained on a single sample of alfalfa meal, and further, there was no reasonable explanation for the big discrepancy in the results obtained by the different research groups. Further data on different lots of alfalfa of known history were needed.

This year the Western Regional Research Laboratory set up further work at the Wisconsin Alumni Research Foundation. The ADA and the University of Illinois again collaborated. Dr. H. M. Scott, who was unable to be here, has kindly permitted me to present his part of the data which are shown in Figures 1 and 2. The first of these shows the liver tocopherols obtained when increasing levels of d- α -tocopheryl acetate were fed. You will note that there is a correlation although the results are somewhat erratic. The dip at the highest level is probably not significant but it does raise a doubt as to whether extrapolation is warranted. In Figure 2 the percent utilization of alfalfa E at the different levels fed is shown. It is clear that at low levels alfalfa vitamin E is used even better than the pure acetate. As levels are increased, however, utilization efficiency drops to below 100%. This should be repeated but it does point to a possible source of the variation between laboratories. I do not believe it explains all of the difference, however, since at the levels used in the Connecticut work they should have gotten over 100% availability.

In Figure 3 are shown the results of the Western Regional Laboratory work on alfalfa as such and treated in three different ways compared with d- α -tocopheryl acetate. In the data shown a 20% protein meal was used. One lot was pelleted and reground. This had no effect on the E content. Other batches were treated with Santoquin and 1% or 5% of fat. The tocopheryl acetate was obviously not utilized any better than alfalfa tocopherol. I do not know why the pellet low level gave such high liver storage. Since the other pelleted samples were not as high, it seems possible that this would not be repeatable. It seems likely from the data that use of Santoquin produced higher absorption at low levels. The interesting but unexplainable part of the data is the fact that higher levels in each case resulted in lower storage per mg. added to the ration. These results are so consistent in our data that it seems likely that it is real. Two other lots of alfalfa meal containing 15% and 23% protein gave comparable results. In all six bioassays, alfalfa tocopherol was at least as well utilized as α -tocopheryl acetate based on liver storage.

It is interesting to compare these results with vitamin A liver storage on the same birds (Figure 4). We see that the reground pellets were about the same as the meal. Adding Santoquin, however, increased vitamin A in the livers. Again we see the higher levels lower than the lower levels. This is apparently an expression of the law of diminishing returns.

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Where does all of this leave us insofar as vitamin E is concerned?

I believe some of the questions have been answered. Thus we know fairly well how much vitamin E is in quite a series of alfalfa samples. Our data indicate that alfalfa tocopherol is essentially all available at the levels which would ordinarily be used. What we do not know is why two groups of investigators do not agree on availability. It was pointed out that analytical differences might be a contributor and that the effect of feeding levels on liver storage might affect interlaboratory checks. It seems likely, however, that these effects could not explain the discrepancies. It was thought that pelleting, fat additions or antioxidant might affect the results but these do not reconcile the intralaboratory results. The only thing I can think of now is that Singen and Bunnell used very low E alfalfa (Table 1). Their samples were so low in E that they must have aged for a considerable period of time. Perhaps the E in such alfalfa meal is actually less available than that in fresh meal or perhaps the analytical method is not suitable for such old samples. Further work should be done to check this point but until it is done I shall assume that this suggestion is valid. This leaves the alfalfa industry in a fairly good position insofar as E is concerned if they can produce a really high quality product so as to meet the animal's requirements with low levels of alfalfa feeding. The other line of research which should be done is to re-evaluate the requirements of various types of poultry for vitamin E with adequate levels of antioxidant and unidentified growth factors in the ration. I feel that when the suggested research is completed it is very likely that upgraded meal with high vitamin E content will be a legitimate vitamin E concentrate and should command concentrate prices.

Table 1

Vitamin E Content of Dried Alfalfa

<u>Reference</u>	<u>Samples</u>	<u>Method</u>	<u>I.U./Lb.</u>
Brown (1953)	"Dried" 4 varieties	Chromatographic	149 - 189
Cabell & Ellis (1942)	"Alfalfa leaf meal"	Rat assay	259
Wall & Kelly (1946)	"Alfalfa leaf meal"	Chem. F.M.	155
" " "	" " "	Chem. E.E.	176
Singsen, et al. 1955	"Alfalfa meal"	Chem. E.E.	49.7 - 69.0
Bunnell (1957)	"Alfalfa meal"	Chem. E.E.	55.0
Pyke, et al. (1957)	Dehy. 100,000 I.U. carotene	" "	89-135
" " " "	" 150,000 I.U. carotene	" "	116-159
" " " "	" 200,000 I.U. carotene	" "	147-192
{Singsen-Bunnell (1956)	Dehy. check samples A.B.C.	" "	155-128-100
{Derse (W.A.R.F.) (1956)	" " " "	" "	112-105-89
{Harris (D.P.I.) (1956)	" " " "	" "	116-100-83
{Harris (D.P.I.) (1958)	15% prot. dehy. W-2		98
{Harris (D.P.I.) (1958)	20% prot. dehy. W-3		122
{Harris (D.P.I.) (1958)	23% prot. dehy. W-4		114
{Harris (D.P.I.) (1958)	20% prot. pellets W-15		113
{Derse (W.A.R.F.) (1958)	15% prot. dehy. W-2	Chem. E.E.	110
" " " "	20% prot. dehy. W-3	" "	103
" " " "	23% prot. dehy. W-4	" "	147
" " " "	20% prot. dehy. pellets W-15	" "	107
Derse (W.A.R.F.) (1958)	20% prot. dehy. pellets		
" " " "	w/Santoquin 1% fat	" "	110
" " " "	20% prot. dehy. pellets		
" " " "	w/Santoquin 5% fat	" "	92
D.P.I. (1956-58)	"Good quality alfalfa meal"	" "	149

Table 2

Poultry Requirement, Recommendation
or Amount Which Gave Protection

<u>Reference</u>	<u>Level (I.U./Lb.)</u>	<u>Comment</u>
<u>Growing Chickens</u>		
Bird, et al. (1940)	1.7	Edema
Dam, et al. (1938)	1.7	Encephalo.
Grau, et al. (1949)	2.0	"
M. L. Scott, et al. (1955)	7 - 14	Diathesis
Singsen, et.al. (1953)	0 - 5 9 - 13	No stress - encephalo. Stress - encephalo.
Patrick & Morgan (1943)	2.0	Growth stimulation
Scott (1958)	7.0	Al.
<u>Chicken Breeder Hens</u>		
M. E. Coates (1949)	1.85	Customarily added
Dju, Quaife, & Harris (1950)	7.2	Total req. for egg storage
M. L. Scott (1958)	10	Hatch & Fert.
<u>Turkey Growth</u>		
M. L. Scott (1952-3)	7.2	Enlarged hock
Slinger, et al. (1954-5)	5	" "
M. L. Scott (1958)	16	" "
<u>Turkey Breeders</u>		
Scott & Nelson (1955)	27 6.8	Added Necessary when 2.5% grass juice was added.
Jensen, et al. (1955)	20	Added - not req.
Atkinson, et al. (1954)	27	Added
M. L. Scott (1958)	25	Total reproduction

Table 3.

Vitamin E Content of a
Typical Poultry Growing Ration

	Vitamin E		
	<u>Lb./T.</u>	<u>I.U./Lb. of Ingredient</u>	<u>I.U./T. of Feed</u>
Alfalfa leaf meal	60	149	8,900
Yellow corn meal	920	2.7	2,500
Soybean oilmeal (50%)	690	0.6	410
Dried whey	50	.16	8
Distillers solubles	50	--	--
Tallow	100	1.8	182
Miscellaneous	130	--	--
			<u>12,000</u>

Table 4.

Vitamin E Content of a Typical Breeder Ration

	Vitamin E		
	<u>Lb./T.</u>	<u>I.U./Lb. of Ingredient</u>	<u>I.U./T. of Feed</u>
Alfalfa leaf meal	140	149	20,900
Yellow corn meal	900	2.7	2,400
Soybean oilmeal	500	0.6	300
Barley	300	3.4	1,000
Miscellaneous	160	--	--
			<u>24,600</u>

Table 5.

Combined Data of Two Experiments Comparing
Two Sources of Vitamin E: D-Alpha Tocopheryl
Acetate and Alfalfa Meal (D-Alpha-Tocopherol)

Treatment	I. U. Vit. E.	d-alpha-tocopheryl acetate		Alfalfa meal	
		% Mort.	Pos. EM.	% Mort.	Pos. EM.
Positive Control	0	0	--	0	--
Negative Control	0	55	17/27*	--	--
	1	37	5/7	--	--
	1.5	--	--	39	6/7
	2	22	6/8	32	3/7
	3	--	--	47	9/9
	4	5.5	2/2	20	3/3
	6	--	--	50	6/9
	8	0	0/0	8	1/2

* Numerator of the fraction is the number of positive cases of encephalomalacia, and the denominator is the number of brains examined.

Table 6.

Plasma and Liver Values for Vitamin E from Chicks Fed
Various Levels, from Different Sources, of Equimolar Equivalents
to dl-Alpha-Tocopheryl Acetate. Average of 3 Replicates.

Equimolar equivalent of mg./ lb. of dl-alpha-tocopheryl acetate.	2	4	8	16
dl-alpha-tocopheryl acetate				
Liver tocoph., mcg./g.	1.86	3.09	4.79	8.40
Plasma tocoph., mg. %	.0280	.1159	.3289	.5452
d-alpha-tocopheryl acetate:				
Liver tocoph., mcg./g.	2.30	3.56	6.38	12.26
Plasma tocoph., mg. %	.3155	.2957	.5649	.5664
dl-alpha-tocopherol:				
Liver tocoph., mcg./g.	2.09	2.96	4.47	8.65
Plasma tocoph., mg. %	.0929	.1993	.2802	.4723
d-alpha-tocopherol from alfalfa:				
Liver tocoph., mcg./g.	1.64	2.51	3.32	5.06
Plasma tocoph., mg. %	.0652	.2289	.3210	.3612

Table 7.

Liver Tocopherol Stores of Chicks After 21
Days on the Assay Diets
(mcg./g. fresh liver)

d- α -tocopheryl acetate			: Alfalfa leaf meal	
4	:	8	:	16
I. U./lb.	:	I. U./lb.	:	I. U./lb.
5.46		6.88		12.63
4.82		6.86		14.47
4.61		5.86		13.06
4.25		8.47		13.76
4.85		7.13		11.71
4.91		7.68		12.88
4.82		6.67		12.08
3.85		7.77		16.79
avg. 4.696		7.165		13.422

Table 8.

Preliminary Experiment 1955*

	4 Wk. Wts.	Liver Analyses	
		Vitamin A (mg./gm.)	Tocopherols (mg./100 gm.)
Basal	339	560	5.75
+ 5% alfalfa meal	386	785	5.94
+ d. α -tocopheryl acetate ~5% alf. meal	359	604	5.87
+ d. α -toco. and 5% alf.	363	710	6.31

* Basal ration held in refrigerator and alfalfa meal added three times per week.

Day-old White Rock males - No symptoms.

Table 9.

Summary of Phase I Results (W.A.R.F.) 1956*
Connecticut Ration

	No. <u>Survivals</u>	27 Day Weights (gm.)	Liver** Tocopherol (mg. %)	Tocopherols per Liver (mg.)
Basal Ration	19	344	3.85	0.437
+ 2 1/2% alfalfa meal	20	386	4.52	0.574
+ d. α -tocopheryl ace- tate ~ 2 1/2% alf. meal	20	391	4.45	0.467
+ 5% alfalfa meal	19	401	5.27	0.645
+ d. α -tocopheryl ace- tate ~ 5% alf. meal	20	395	4.41	0.493

* Each figure represents the average of duplicate groups of ten chicks each.

**Severich and Baumann, Anal. Chem., 24: 758 (1952); Parker and McFarlane, Can. J. Res., B, 18, 405 (1940); Quaife and Harris, J. Biol. Chem. 156: 499 (1944).

Table 10.

Summary of Phase II Results (W.A.R.F.) 1956*
Connecticut Ration

	No. <u>Survivals</u>	27 Day Weights (gm.)	Liver Tocopherol (mg. %)	Total Tocopherols per Liver (mg.)
Basal Ration	11	193	5.04	0.361
+ 2 1/2% alfalfa meal	15	253	5.61	0.502
+ d. α -tocopheryl ace- tate ~ 2 1/2% alf. meal	18	236	4.70	0.326
+ 5% alfalfa meal	19	237	5.74	0.458
+ d. α -tocopheryl ace- tate ~ 5% alf. meal	18	240	5.29	0.432

* Each figure represents the average of duplicate groups of ten chicks each.

Table 11.

Summary of Phase III Results 1956*
University of Illinois

	<u>No. Survivals</u>	<u>27 Day Wts. (gm.)</u>
Basal Ration	20	165
+ 2 1/2% alfalfa meal	20	191
+ d. α -tocopheryl acetate ~ 2 1/2% alfalfa meal	18	164
+ 5% alfalfa meal	17	184
+ d. α -tocopheryl acetate ~ 5% alfalfa meal	20	185
+ 3% A + D feeding cod liver oil	10	143

Note: Neither encephalomalacia nor exudative diathesis was observed in any of the chicks. Ataxia from arginine deficiency was common to all lots.

* Each figure represents the average of duplicate groups of 10 chicks each.

Table 12.

Results of Biological Study on the
Vitamin E Content of Alfalfa Meal - Cornell 1956

	<u>Survivors*</u> No.	<u>Wt.</u> Gms.	<u>Ex.</u> No.
Basal (Torula yeast)	3/15	141	10
3 mg. E (4.1 I.U.)	15/15	258	6
5 mg. E (6.8 I.U.)	12/14	268	5
6.25% Alfalfa meal (equiv. to 6.8 I.U.)	14/15	295	4
12.5% Alfalfa meal (equiv. to 13.6 I.U.)	15/16	294	0

*No. survivors 4 weeks
No. survivors 2 weeks

Table 13.

Cornell Experiment - 1956

<u>Treatment*</u>	Avg. wt. <u>3 Weeks</u> gm.	Exudates <u>3 Weeks</u> %
Torula basal diet	152	84
3 mg. d. α -tocopheryl acetate (E) (4.08 I.U./lb.)	176	7
5 mg. E (6.8 I.U./lb.)	191	0
6.25% alfalfa meal (7.2 I.U./lb.)	214	6
12.5% alfalfa meal (14.4 I.U./lb.)	202	0

* DPPD, 100 mg./lb., added to all diets.

Figure 1. H.M. Scott Data 1958

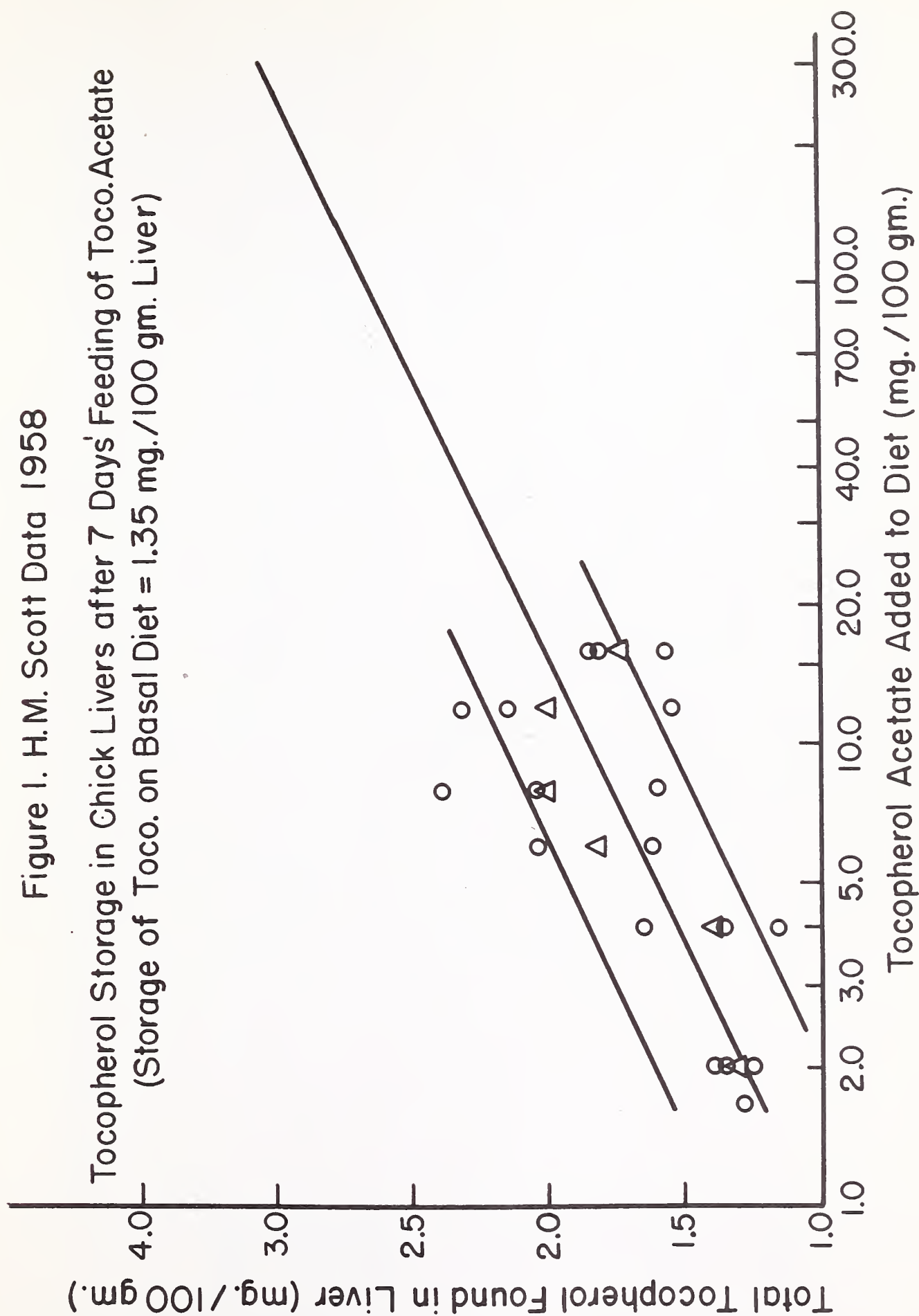


Figure 2. H.M. Scott Data 1958

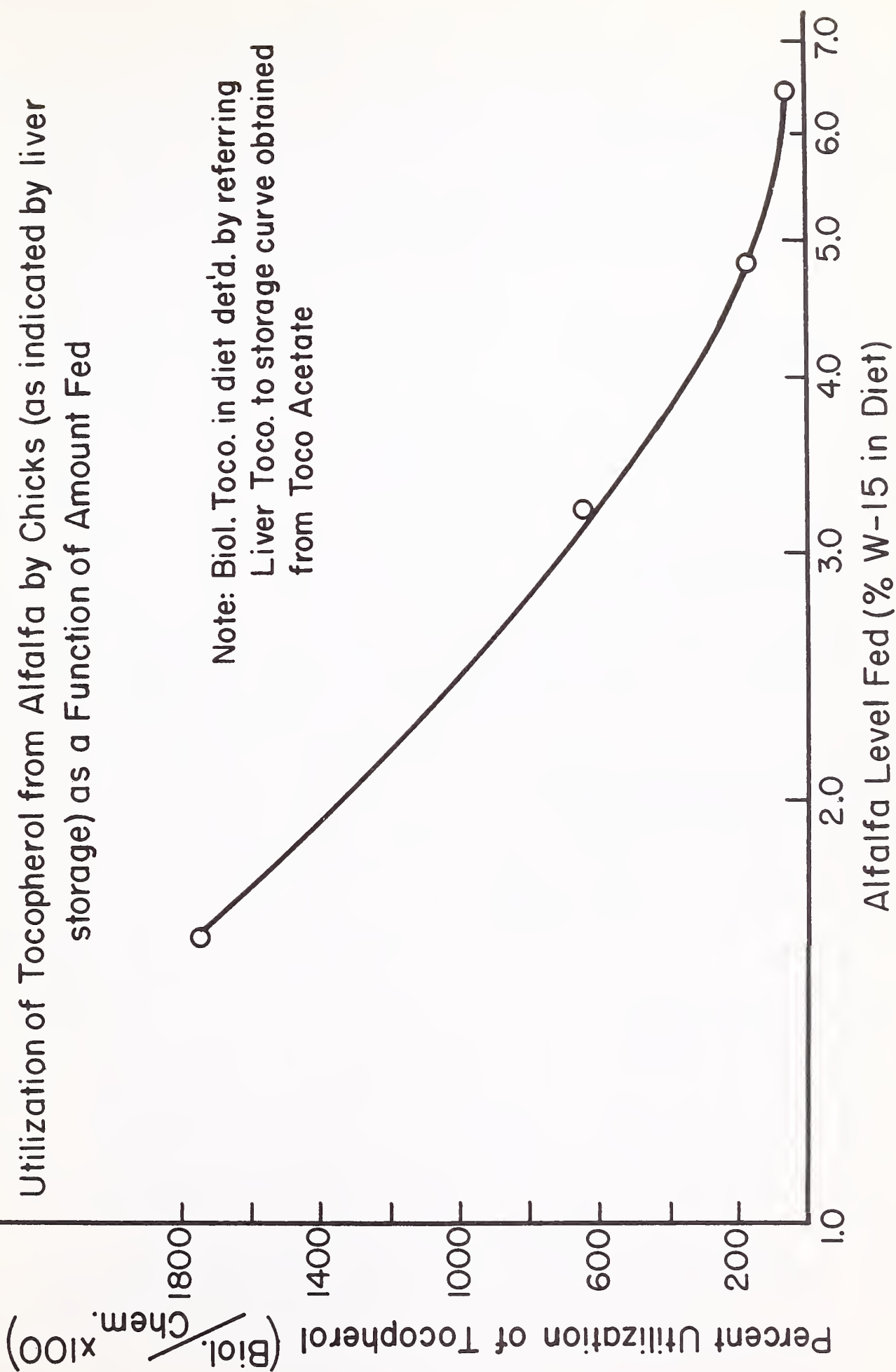


Figure 3. W.A.R.F. 1958

Storage of Tocopherol in 4-Week Chick Livers as Function of Processing
of Alfalfa and as Function of Level of Alfalfa in Diet
(Storage of Toco. on Basal Diet = 1.82 mg./100 gm. Liver)

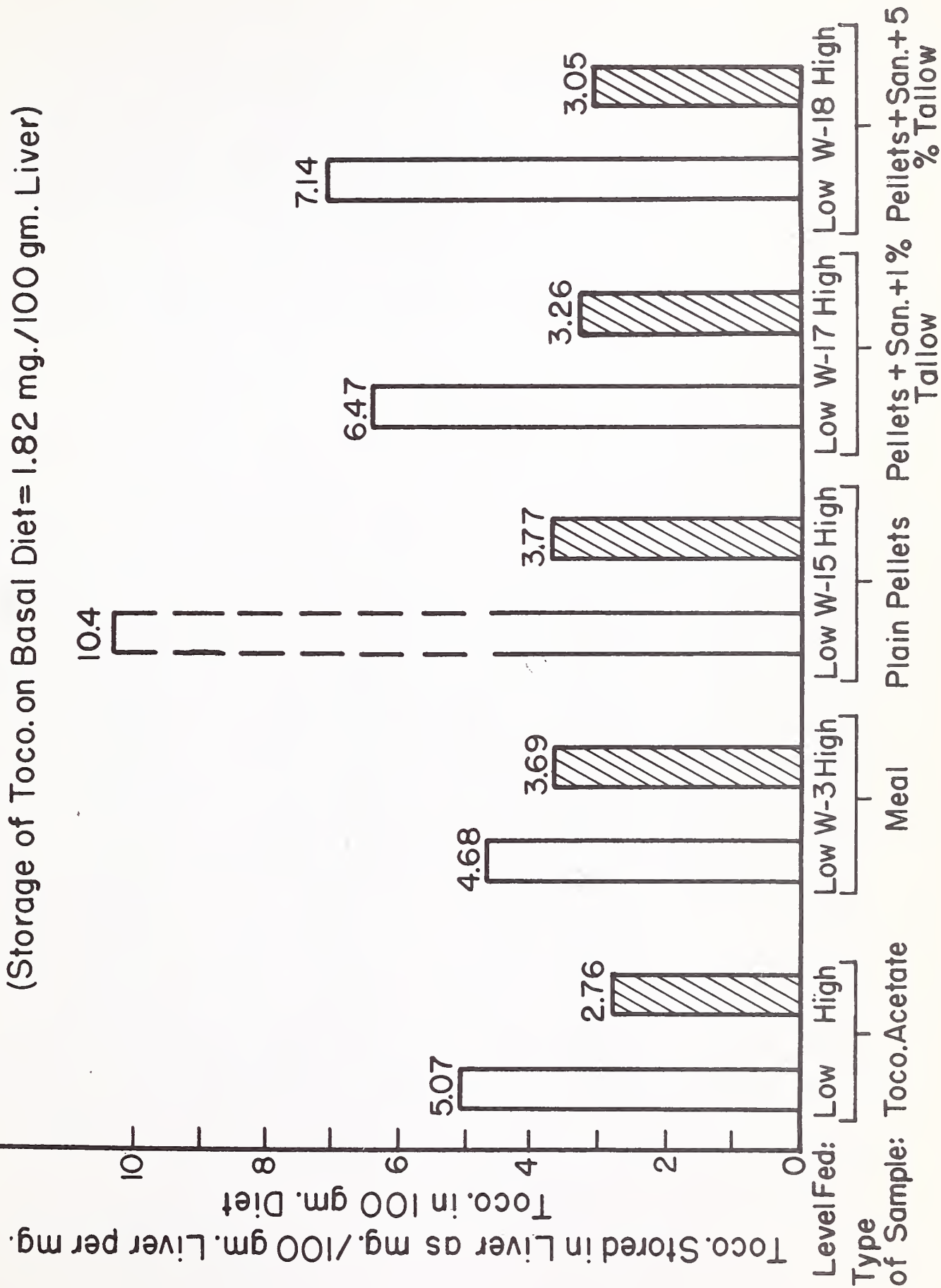
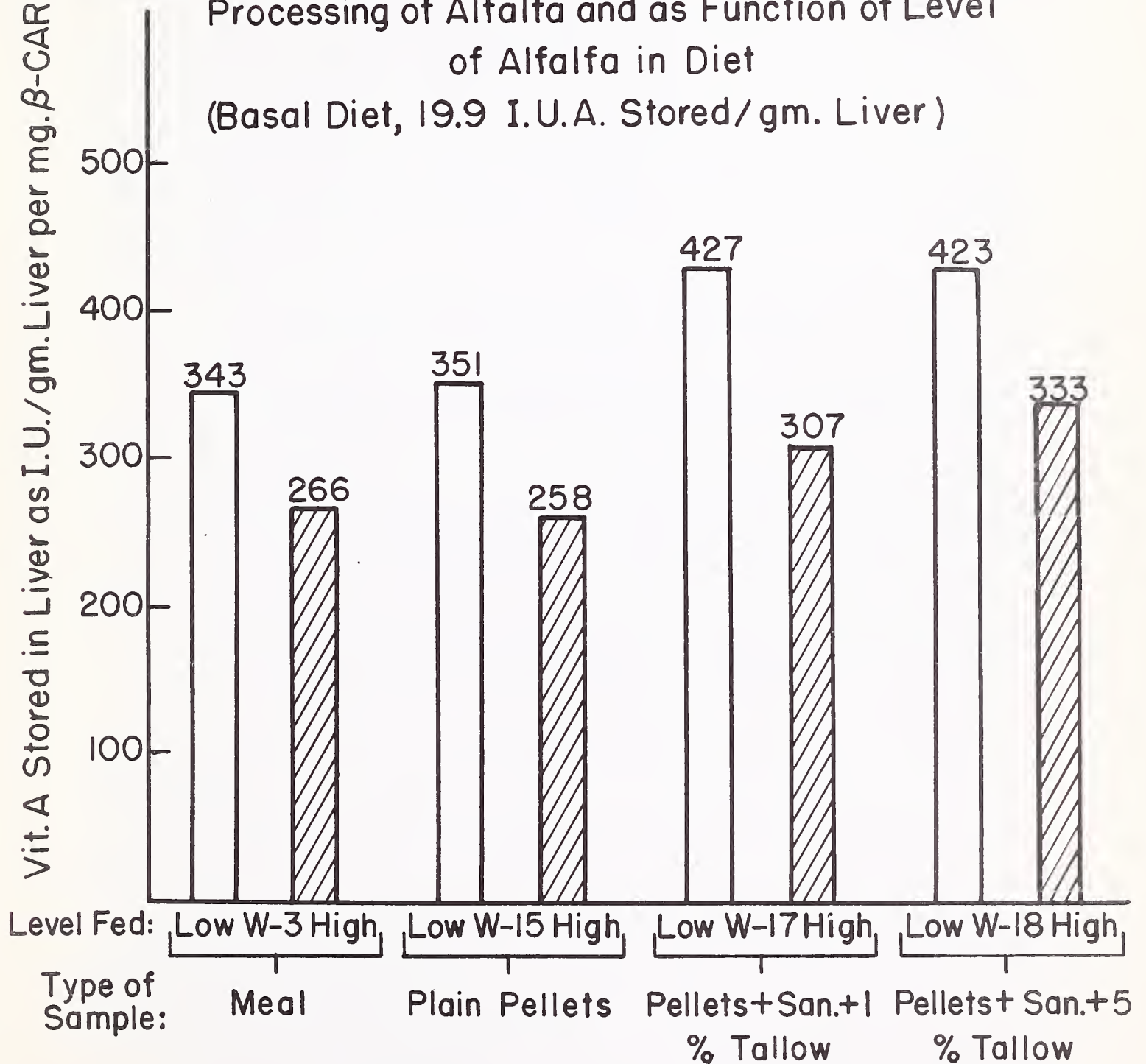


Figure 4. W.A.R.F. 1958

Storage of Vitamin A in 4-Week Chick Livers as Function of Processing of Alfalfa and as Function of Level of Alfalfa in Diet

(Basal Diet, 19.9 I.U.A. Stored/gm. Liver)



✓ Dehydrated Alfalfa As A Protein Supplement

(with or without stilbestrol) For Fattening Cattle 1/

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A marked response in cattle performance, particularly in appetite, came to our attention in the summer of 1955. An experiment which was being conducted at Nebraska involved the comparison of various protein supplements (with and without stilbestrol). Slide one and two present a summary of two tests. Slides three and four present the combined data for the two trials. In view of these findings an experiment was started in the spring of 1957 to determine the value of the following protein supplements when fed with or without stilbestrol in fattening beef cattle rations:

- (a) soybean oil meal, the level adjusted according to the additional needs of protein in the ration
- (b) dehydrated alfalfa meal, to furnish a similar amount of protein as supplied by the quality of soybean oil meal fed in (a)
- (c) combination of soybean oil meal and dehydrated alfalfa meal, approximately half the amount of protein supplied in (a) to be furnished by soybean oil meal and the other half by dehydrated alfalfa meal
- (d) dehydrated alfalfa meal, fed at twice the level used in (b), recognizing that animals in this group would receive twice as much protein from the supplement.

The Experiment

Cattle Used

Eighty yearling steers of "good to choice" quality were divided into eight lots.

Prior to this the animals had been used in wintering tests. None of the animals previously had been fed stilbestrol nor treated with stilbestrol implants or similar material. During the wintering tests, the calves had gained approximately 1.5 pounds per head daily.

^{1/}Major portion of the material contained in this report was taken from the Nebraska Agriculture Experiment Station 46th Annual Feeders Day, April 18, 1958, Cattle Progress Report 250.

^{2/}Present address: Director of Experiment Station, Burlington, Vermont.

Feeds Used

No. 2 grade, ground, shelled corn was full-fed. The solvent-processed soybean oil meal had a minimum protein guarantee of 46 per cent. Fine ground dehydrated alfalfa meal had a minimum protein guarantee of 17 per cent but no guarantee on vitamin A content. Prairie hay which contained approximately 5.2% protein was fed at approximately the same rate to all lots.

Results

The experiment extended over a period of 155 days -- May 24, 1957 to October 26, 1957.

The eighty head of yearling steers were fed in eight lots of ten head each. The animals were fed all the ground shelled corn they would clean-up within a period of two to three hours after each feeding (the cattle were fed twice daily). Prairie hay was limited to about 3.5 to 4 pounds per head daily. A non-legume hay was used to keep the protein content and also the possibility of estrogenic substances to a minimum. The protein supplements and the amounts fed in the daily ration for the major part of the feeding test are shown on slide 5.

The effect of various supplements (with or without stilbestrol) on the daily gains and efficiency of feed utilization are summarized on slide 6. The largest gains were made by steers fed the 2-X level (4.5 lbs. daily) of dehydrated alfalfa meal with stilbestrol added to the ration. The steers fed 1-X level (2.3 pounds daily) of dehydrated alfalfa meal with stilbestrol made the second largest gains and made more efficient gains than the steers fed the higher level of dehydrated alfalfa meal. The smallest gains were made by the steers fed soybean oil meal as the protein supplement and without stilbestrol.

The per cent increase in steer gains and feed efficiency are shown on slide 7.

Feeding of 10 milligrams of stilbestrol in the daily ration to the four lots of steers increased gains 15.6 per cent and feed efficiency 10.9 per cent. Of the protein supplements compared in this experiment, the addition of stilbestrol gave the greatest response to the ration which contained soybean oil meal. However, steers fed soybean oil meal with stilbestrol added to the ration gained an average of only 6 pounds more per head during the 155-day feeding period as compared to the steers fed the 1-X level of dehydrated alfalfa meal without stilbestrol. This indicates the potential estrogenic activity or perhaps the presence of some unknown factor in dehydrated alfalfa. The lower gains by steers fed the 2-X level of dehydrated alfalfa meal (without stilbestrol) may be due in part to the decreased grain consumption.

A summary of slaughter and carcass data are shown on slides 8 and 9. All of the steers were sold at the Omaha Stock Yards for the same price per hundred weight. There were no great differences in carcass grades in these comparisons. No visual differences were found in color of fat, marbling, or in carcass conformation.

A general summary of the weight gains, daily ration and the feed requirement per hundred weight gain for each of the lots are shown in the following table.

Slide 1.

Protein Supplements with D.E.S. at Different Periods

Nebraska 1955 Trial I - 112 days

	S.O.M.	LSOM	Dehy.	S.O.M.
Daily ration, lb.:				
Grd. sh. corn	20.9	20.4	20.9	
Supplement	.73	1.0	.5 and .75	
Alfalfa hay	2.9	3.2	2.9	
Prairie hay	2.0	2.1	2.1	
Avg. daily gain, lb.	3.02	3.12	3.20	
Feed efficiency, lbs.	880	854	850	

Slide 2.

Protein Supplements with D.E.S. at Different Periods

Nebraska 1956 Trial II - 112 days

	S.O.M.	LSOM	Dehy.	S.O.M.
Daily ration, lb.:				
Grd. sh. corn	18.0	17.9	17.8	
Supplement	.75	1.0	.5 and .75	
Alfalfa hay	3.8	3.8	3.9	
Avg. daily gain, lb.	2.40	2.62	2.65	
Feed efficiency, lbs.	938	868	864	

Slide 3.

Protein Supplements with D.E.S. at Different Periods

Nebraska 1955 and 1956 112 days

	S.O.M.	LSOM	Dehy.	S.O.M.
No. steers	36	33	36	
Initial weight	985	994	984	
Avg. daily gain	2.71	2.87	2.93	
Feed/cwt. gain	909	861	857	
Dressing %	62.7	62.8	62.9	
Carcass:				
Choice	23	25	29	
Good	13	10	6	

.75# S.O.M.; 1.0# LSOM; .75# Dehy. .5# S.O.M.

Slide 4.

When Should Stilbestrol be Added?

Nebraska Two Trials

112 day feeding.

Initial weight 985 lbs.

(controls 2.65 lbs. gain)

Stilbestrol

	1st HALF	2nd HALF	ENTIRE	AVERAGE
S.O.M.	2.71	2.77	2.66	2.71
Linseed	2.88	2.90	2.84	2.87
S.O.M. plus Dehy.	2.89	2.86	3.03	2.93
AVERAGE	2.83	2.84	2.84	

Slide 5.

Dehy. Alfalfa (with or without D.E.S.)
Supplements Fed Daily

Nebraska 1957 - Trial I

Lot No. 1/	1 & 5	2 & 6	3 & 7	4 & 8
Avg. for feeding period: <u>2/</u>				
Dehy. alfalfa, lbs.	2.3	0	1.2	4.5
S.O.M., lbs.	0	.8	.5	0

When cattle adjusted to supplements:

Dehy. alfalfa, lbs.	2.5	0	1.25	5.0
S.O.M., lbs.	0	.8	.5	0

1/ Lots 1, 2, 3 and 4 were fed D.E.S., 10 mg. daily/head
2/ Lots 1, 4, 5 and 8 reduced in dehy. last 17 days..

Slide 6.

Dehy. Alfalfa (with or without D.E.S.)

Nebraska 1957 Trial I

<u>Supplement</u>	<u>No Stilbestrol</u>		<u>With Stilbestrol</u>	
	<u>Gain</u>	<u>Feed/cwt.</u>	<u>Gain</u>	<u>Feed/cwt.</u>
S.O.M.	1.86	1058	2.51	853
1X Dehy.	2.47	901	2.61	879
2X Dehy.	2.23	1018	2.64	894
Dehy. S.O.M.	2.38	891	2.59	860
Average	2.24	967	2.59	872

Slide 7.

Dehy. Alfalfa (with or without D.E.S.)

Nebraska 1957 Trial I

	<u>% Increase in</u>	
	<u>Gain</u>	<u>Feed Efficiency</u>
	<u>None</u>	<u>D.E.S.</u>
1X Dehy. vs. S.O.M.	32.8	4.0
2X Dehy. vs. S.O.M.	19.9	5.2
1X Dehy. vs. 2X Dehy.	10.8	-1.1
1X Dehy. vs. Comb.*	3.8	0.8
Comb. vs. S.O.M.	28.0	0.3
Comb. vs. 2X Dehy.	6.7	-1.9

*Comb. = Dehy. Stilbestrol

Slide 8.

Dehy. Alfalfa (with or without D.E.S.)

Nebraska 1957 Trial I

<u>Carcass Grades</u>	<u>S.O.M.</u>	<u>1X Dehy.</u>	<u>2X Dehy.</u>	<u>Comb.</u>
High Choice,%	40	70	50	35
Avg. Choice,%	45	20	30	30
Low Choice,%	0	0	0	5
High Good,%	0	0	0	0
Avg. Good,%	10	10	20	30
Low Good,%	5	0	0	0
Dressing %	61.6	61.8	62.1	62.1

Slide 9.

Dehy. Alfalfa (with or without D.E.S.)

Nebraska 1957 Trial I

Lot No.	Stilbestrol					No Stilbestrol		
	1	2	3	4	5	6	7	8
Carcass Grades:								
High Choice	8	2	4	5	6	6	3	5
Avg. Choice	1	7	2	4	3	2	4	2
Low Choice	0	0	1	0	0	0	0	0
High Good	0	0	0	0	0	0	0	0
Avg. Good	1	1	3	1	1	1	3	3
Low Good	0	0	0	0	0	1	0	0

General Summary of Feeding Experiment
May 24, 1957 to October 26, 1957 -- 155 days
(Data based on one average steer per lot)

Lot Number	1	2	3	4	5	6	7	8
No. steers per lot	10	10	10	10	10	10	10	10
Avg. initial weight, lbs.	694	695	695	697	695	696	690	698
Avg. final weight, lbs.	1099	1084	1096	1106	1078	985	1059	1043
Avg. gain/head, lbs.	405	389	401	409	383	289	369	345
Avg. daily gain/head, lbs.	2.61	2.51	2.59	2.64	2.47	1.86	2.38	2.23
Average daily ration per head, lbs.:								
Ground shelled corn	17.2	17.2	17.2	15.6	16.4	15.8	16.2	14.6
Dehydrated alfalfa meal	2.3	--	1.2	4.5	2.3	--	1.2	4.4
*Soybean oil meal	.06	.8	.5	.06	.06	.8	.5	.06
Prairie hay	3.4	3.4	3.3	3.4	3.5	3.2	3.3	3.6
Stilbestrol, mg.	10	10	10	10	--	--	--	--
Total feed per head, lbs.:								
Ground shelled corn	2667	2673	2670	2425	2541	2443	2514	2268
Dehydrated alfalfa meal	356	--	187	691	356	--	186	683
*Soybean oil meal	9	122	76	9	9	121	76	9
Prairie hay	528	524	514	532	546	494	513	551
Stilbestrol, mg.	1525	1525	1525	1515	--	--	--	--
Feed consumed per 100 pounds live weight gain, lbs.:								
Ground shelled corn	659	687	666	593	663	845	681	657
Dehydrated alfalfa meal	88	--	47	169	93	--	50	198
*Soybean oil meal	2	31	19	2	2	42	221	3
Prairie hay	130	135	128	130	143	171	139	160
Stilbestrol, mg.	377	392	380	370	--	--	--	--
Total feed, lbs.	879	853	860	894	901	1058	891	1018

*Soybean oil meal was fed in lots 1, 4, 5 and 8 during the last 17 days when dehydrated alfalfa was reduced.

X FEEDING PELLETED ALFALFA TO LAMBS

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Advantages of feeding completely pelleted high roughage rations to lambs are: (1) Reduced storage space, (2) Better adapted to mechanical handling of feed, (3) Less wasted feed, (4) Complete consumption of ration as prepared, (5) Improved palatability, (6) Easier to start lambs on feed, (7) Increased feed consumption, (8) Quality of roughage may not be as important, (9) Less death loss, (10) Increased gains, (11) Less feed per pound of gain, (12) Higher dressing percentage, (13) Better carcass grading, and (14) Can be self-fed with less feeder space needed. The major disadvantages of the pelleted rations are: (1) Cost of processing and pelleting, and (2) Ruminant parakeratosis is apparently caused by completely pelleted rations.

Lambs can be fattened when self-fed a ration of either pelleted alfalfa hay or pelleted dehydrated alfalfa. In two experiments, pelleted dehydrated alfalfa gave the largest and cheapest gains. Gains of 0.3 to 0.5 pound per lamb per day with 10 to 17 pounds of feed per pound of gain can be expected.

Pelleted rations consisting of 60 percent alfalfa hay and 40 percent grain when fed to lambs have given the largest gains. Pelleted rations with more alfalfa have given about the same gains as 100 percent alfalfa. Pelleted rations with less than 60 percent alfalfa have not consistently improved gains.

Corn-cob-meal and barley have given slightly larger gains than corn or milo when fed in pelleted rations consisting of 50 percent alfalfa hay and 50 percent grain. Pelleted alfalfa hay has given slightly larger gains in two tests than dehydrated alfalfa with about the same amount of feed needed to produce a pound of gain when fed in pelleted rations consisting of 50 percent alfalfa and 50 percent milo. The addition of ground limestone or dicalcium phosphate to pelleted rations has materially improved efficiency of gains. One milligram of stilbestrol per pound of pelleted feed improved gains by 0.1 of a pound per lamb per day with marked improvement in efficiency in one experiment.

The grind of alfalfa is a very important factor in the feeding value of/pelleted ration. The coarser grind seems to improve rate and efficiency of gain. This problem requires more study. Lambs can use large pellets as well as small pellets providing the pellets are not too hard. One group of lambs fed a wafer-type pellet measuring 1 inch x 1 inch x 1.25 inch gained as well as similar group of lambs fed the same ration in one-fourth inch round pellets.

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EVALUATION OF XANTHOPHYLL SOURCES FOR PIGMENTATION
OF BROILERS AND EGG YOLKS

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Consumer demand for fivers with a desirable degree of yellow pigmentation has focused the attention of broiler producers on sources of such pigmentation. In some localities the processor often pays from one to two cents less per pound for birds which are poorly pigmented. It is easy to figure the economic loss to the producer who has birds with inadequate pigmentation.

Not only the broiler producer is concerned with pigmentation, but the egg producer and the consumer are also aware of its importance. Xanthophyll is the main source of yellow pigmentation in the beak, skin, and shanks of broilers, and in the yolk of the egg. Egg noodle manufacturers and other consumers pay premium prices for eggs with properly pigmented yolks.

Xanthophylls are a group of carotenoid pigments which are widely distributed in nature. Structurally, these compounds are related to the carotenes. The most important of these pigments, in so far as broiler and yolk pigmentation are concerned, is lutein. It was pointed out by Fritz (1957) that when the term xanthophyll is used in the singular form, it is synonymous with lutein. It is this compound which poultry producers consider extremely important.

Sources of xanthophyll in poultry feeds depend upon yellow corn, corn gluten meal, alfalfa or added xanthophyll concentrates. The amount of pigmentation present in the broiler is obvious when one observes the shanks of the bird. The amount of pigmentation is related to the amount of xanthophyll in the diet, but this may also be affected by other factors such as disease, heredity, or other feed ingredients.

Culton and Bird (1941) reported a pigment inhibiting factor in meat scraps, fish meal and soybean meal which prevented the deposition of pigment in the shanks. They observed a direct relationship between skin color and shank color. Hammond and Harshaw (1941) found that the amount of pigmentation was dependent upon the level of xanthophyll in the diet. They also observed a pigment depressing factor due to the presence of cod liver oil in the diet. The general feeling today is that fats in the diet may have the same effect that the above authors reported for cod liver oil.

The pigmentation of egg yolks has been of some concern for many years. Heiman and Carver (1935) developed a "Color Roter" which could be used in grading the degree of color in the yolk. This device has been used by some authors for skin and shank color observations since that time. In 1943, Heiman and Tighe presented a colorimetric method for the determination of pigmentation. These authors also reported that pigmentation increased with age and suggested that pigmentation was a gradual process and could not be accomplished quickly. However, Bird (1943) observed that pigmentation in Barred-Rock and New Hampshire chicks could be accomplished within ten days.

Collins, Thayer and Skoglund (1955) reported on breed differences in pigmentation. New Hampshire broilers were significantly darker than White Plymouth Rock chicks, with Barred Plymouth Rocks intermediate between the two. These authors also found significant differences between strains. Hammond and Harshaw (1941) were unable to pigment Light Sussex chicks while White Wyandottes, Rhode Island Reds and White Leghorns could be.

In order to prevent oxidation of certain desirable feed components, poultry feeds usually contain a source of an antioxidant. The relationship of antioxidants to pigmentation has been considered by several investigators. High, et al. (1952, 1954) reported that a high ratio of an antioxidant to carotene in the diet resulted in a decrease in the amount of vitamin A in the liver of rats, and that a low ratio of these components resulted in an increase in liver vitamin A in rats. DPPD, formerly used as an antioxidant in feeds, was reported by Potter et al. (1956) to increase skin carotenoids. Similarly, vitamin B₁₂ (12 mg./ton) and DPPD plus B₁₂ resulted in an increase in the deposition of skin carotenoids. Harms et al. (1958), however, reported that when DPPD was added to diets containing 2½ or 5% dehydrated alfalfa meal, there was a significant decrease in pigmentation. Fritz (1957) reported that the addition of BHT or DPPD resulted in an increase in pigmentation. Williams et al. (1958) have found that BHT has no effect on pigmentation. It seems obvious that the conflicting data reported indicate a need for further study in regard to the effect of antioxidants on pigmentation.

Titus et al. (1938) tested the effect of feeding various substances on yolk color. These authors fed a diet low in pigment to White Leghorn or Barred Plymouth Rock X Rhode Island Red hens. When a low color index of the yolks was obtained, capsules which contained purified leaf xanthophyll, chili pepper, pimento pepper, or acetone, alcohol or petroleum ether extracts were fed to the hens. It was found that the increase in yolk pigmentation was proportional to the xanthophyll in the diet. Similar results were found in relation to the amount of yellow corn in the diet. Xanthophyll at the level of 25 mg./day was essentially equivalent to feeding a diet containing about 80% corn. Variable effects were obtained with the chili and pimento peppers, and the extracts had no effect in most instances. The pigment appeared quickly, as early as the second egg

in some instances, and lasted from 2 to 11 eggs after the pigment supply was removed from the diet. Xanthophyll reached its maximum pigmentation in from 2-6 days. Elrod (1958) found that egg yolk pigmentation was increased as the level of dehydrated alfalfa meal was increased up to 7.5% of the diet.

It should be emphasized that while egg yolks may be made to vary in color due to the dietary constituents, the desirable color is one which is uniform and intermediate in intensity. Undesirable yolks are those which are either too dark or too light.

Fritz (1957) fed Ventress Cross chicks to six weeks of age on a diet low in pigment. When graded levels of xanthophyll were added to the diet, it was found that the addition of 12.5 mg. xanthophyll per lb. of ration produced the average pigmentation found in a conventional fed broiler. Several alfalfa sources were tested in which the best results were obtained with alfalfa pigment granules. Other possible sources of pigmentation were tested and the best results were obtained with dietary additives which were good sources of xanthophyll. Poor results were obtained with the addition of several commercial pigmenters. The author concluded from the numerous and varied tests that xanthophyll was the chief source of pigmentation in the shank, skin and body fat of broilers. None of the other pigments tested were deposited in effective amounts. House (1957) found that from 9.5 to 10 mgs./lb. of xanthophyll were required per pound of feed before the skin color was acceptable.

The amount of xanthophyll present in yellow corn may vary considerably. Various reports range from 8.5 to 16 mgs. xanthophyll per pound (Ferguson 1958). High quality alfalfa meal usually contains about 140 mgs./lb. (Thompson 1957). Other reports of the level of xanthophyll in alfalfa range from 70 to 160 mgs./lb. Corn gluten meal contains about 60 mgs./lb. xanthophyll. At present, the supply of corn gluten meal is rather limited and feed manufacturers are unable to get sufficient quantities.

A relationship between xanthophyll and carotene in dehydrated alfalfa meal has been reported by House (1957) to be 2.1:1. Elrod (1958) reported the relationship of xanthophyll to carotene to be 2.9:1. It is apparent from these and other reports that this factor may vary, but the inert-gas storage of alfalfa tends to preserve the pigments and insures the delivery of a high-potency product. Stabilizers added to the meal aid in preserving the carotene content and serve to protect the xanthophyll to much the same degree (Thompson, 1957).

It is interesting to note that as much as 8 to 10% alfalfa was included in poultry diets 15-25 years ago. The present poultry ration emphasizes energy and its relation to the protein content (calorie-protein ratio). With the development of the high energy ration, a

premium was placed on feed ingredients with low fiber and high energy. As a result, dehydrated alfalfa meal was reduced or left out of the diet. This factor has brought about a revised interest in pigmentation since one of the main xanthophyll sources has been decreased. The increased use of milo in feeds due to its relative abundance and lower cost than corn, has also affected the degree of pigmentation in poultry.

It was pointed out (Ferguson, 1958) that the calorie-protein ratio of a typical broiler diet could be maintained by adding 5% dehydrated alfalfa meal and 1.5% fat in an economical manner, and the amount of pigmentation would be increased by approximately 12 grams. It was also shown that the energy-protein ratio of a diet containing 60% corn could be maintained at the same levels when 27.5% milo, 5% dehydrated alfalfa meal and 1.5% fat were substituted. Furthermore, this change was effected with no increase in cost over the original diet, yet there were 12 grams of xanthophyll (\$9.60) contributed by the alfalfa.

Combs et al. (1958a) fed broilers on diets containing from 2% to 10% fat from both animal and vegetable sources. The diets contained from 1.5-2.5% dehydrated alfalfa meal and from 2.5-5.6% corn gluten meal. The xanthophyll levels ranged from 6.7 to 10.2 mgs./lb. Pigmentation was slightly lower in some groups receiving the high fat diet, but they concluded that 2-10% fat could be used with good results. A second study (Combs et al. 1958b) was conducted under similar conditions. In this instance, dehydrated alfalfa meal ranged from 1.0-2.0%, corn gluten meal from 2.5-5%, and from 2-10% fat. Xanthophyll levels ranged from 7.4-9.4 mgs./lb. Pigmentation scores from all groups were excellent.

It was reported by Elrod (1958) that 20% protein dehydrated alfalfa meal produced better pigmentation than did the 17% protein meal. Five per cent dehydrated alfalfa meal produced the maximum pigmentation with an all milo formula whereas only 2.5 per cent dehydrated alfalfa meal was required for maximum pigmentation with a formula containing corn as the grain source.

The addition of dehydrated alfalfa meal to the poultry ration improves the quality of the diet by the addition of components other than xanthophyll. Alfalfa is sold on a guaranteed basis of 100,000 or 150,000 I.U. of vitamin A potency per pound as carotene, and contains 17 or 20% protein. Kohler (1957) lists the following vitamins per pound of alfalfa: 400 mg. choline, 7 mg. riboflavin, 18 mg. niacin, 16 mg. pantothenic acid, 4 mg. folic acid, 3 mg. thiamine, 6 mg. pyridoxine, 150 mcg. biotin, 950 mg. inositol, 35 mg. vitamin K, 110 mg. vitamin E and 700 mg. of ascorbic acid. Analyses reported by Kohler show a total of 18 amino acids, including all of the essential amino acids. Minerals reported included calcium, phosphorus, magnesium potassium, sodium iron, manganese, copper, cobalt and boron.

Unidentified factors in alfalfa have been reported to improve growth and feed efficiency and aid in normal bone development (Norris and Morrison, 1956; Reid et al., 1956 a,b,c; Couch et al., 1955; Norris, 1955; Scott, 1956; Norris et al., 1956; and Morrison et al., 1956). Thus dehydrated alfalfa meal, in addition to being an excellent and economical source of xanthophyll, contributes protein, vitamins, minerals and unidentified factors which may be utilized by the growing chick and the mature fowl.

SUMMARY

The amount of pigmentation in the skin and shanks of broilers is proportional to the amount of xanthophyll in the diet. The yellow color in the yolk of eggs is also due largely to xanthophyll. Pigmentation in the shanks of the bird will indicate the degree of pigmentation of the skin. Significant differences have been reported in the degree of pigmentation which can be induced between strains, as well as within strains. In birds with a moderate amount of pigment, the addition of a source of xanthophyll to the diet will produce pigmentation within a relatively short time.

The possibility that antioxidants may affect the degree of pigmentation has been reported. Further work should be done in order to answer this question, about which there have been conflicting reports.

The main sources of xanthophyll in feed stuffs are yellow corn, corn gluten meal and dehydrated alfalfa meal. Corn gluten meal is often in short supply. The use of milo in many poultry feeds has required that an outside source of xanthophyll be used, since milo does not contain xanthophyll. High energy rations and a definite calorie-protein ratio are characteristics of today's poultry diet. It has been pointed out that it is economical for one to add 1.5% fat with 5% dehydrated alfalfa meal to a corn-soybean meal diet and maintain the same protein and energy of the original diet. The diet as altered would add about 12 grams of xanthophyll for pigmentation.

It was further pointed out that the corn-soy diet could be changed by the addition of 27.5% milo, 1.5% fat and 5% dehydrated alfalfa meal without any increase in cost, yet maintain the same calorie-protein ratio as the original diet. This provides additional xanthophyll for pigmentation. Some workers have used either 2% or 10% fat and produced birds with excellent pigmentation.

Additional gains from the use of dehydrated alfalfa meal would result since the alfalfa would contribute protein, vitamins, minerals and unidentified factors, as well as the xanthophyll which would be available for shank, skin and yolk pigmentation.

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PELLETING ROUGHAGE, GRAIN OR COMPLETE RATIONS
FOR BEEF AND DAIRY CATTLE

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ABSTRACT

The generally good results with pelleted complete rations for fattening lambs have stimulated active research on the possibilities of pelleted feeds for beef cattle, and to a lesser extent with dairy cattle.

The advantages of pelleting feeds for beef cattle may be summarized as follows:

1. Permits bulk handling of feeds with automatic equipment, providing opportunities for labor savings.
2. Permits self-feeding.
3. Reduces chances for mixing and feeding errors by hired labor; every bite is nutritionally complete.
4. Cuts down feed waste, due to wind, sorting by animals, etc.
5. Increases consumption of poor quality roughages.
6. Usually gives improved feed efficiency; rate of gain of cattle may or may not be increased.

A review of the currently available data on the pelleting of roughages, grain, or complete rations for beef cattle indicates the following: (These interpretations of current results should be considered as preliminary in nature, as they may be altered substantially by the results of research now in progress).

1. The pelleting of either good or poor quality roughages for cattle has resulted in increased rate of gain and improved feed efficiency.
 2. Quality differences between roughages are not completely eliminated by pelleting.
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3. The results of a limited number of experiments indicate that pelleting only the grain portion of the ration for cattle also gives increased rate of gain and improved feed efficiency.
4. The pelleting of complete rations for beef cattle appears to give a consistent and marked improvement in feed efficiency but has a variable effect on rate of gain.
5. Pelleted high roughage rations apparently will not produce a satisfactory degree of fatness or market finish in beef cattle, as contrasted with sheep; high concentrate levels appear to be necessary in pelleted beef cattle rations to produce most rapid gains and acceptable market finish.
6. Current results indicate that the improvement in feed efficiency due to pelleting and the reduction in labor costs generally give savings in total feeding costs sufficient to balance the additional costs of processing and pelleting cattle rations.

Currently, there is a great interest among feed processors, feeders and others in the production of compressed hay pellets, wafers, briquette, etc., as a possible replacement of present hay baling methods. Pelleted or "wafered" hay offers many advantages from the standpoint of possibilities for bulk-handling and storage on farms. The production of hay "wafers" or pellets from coarsely ground or chopped roughage also appears to have definite nutrition value in preventing the drop in butterfat production which occurs when finely ground roughages are fed to dairy cows.

Successful development of equipment to produce hay "wafers" or pellets from coarse-ground or chopped hay could place lower-quality, field-cured roughages in stronger competition with dehydrated alfalfa for ruminant feeding.

Alfalfa dehydrators need to appraise these developments in the light of a need for different methods of processing dehydrated alfalfa for ruminant feeding. It appears that a special coarse ground dehydrated alfalfa may be superior to present fine-ground products for ruminant feeding. More research is needed on this point.

Current developments in the pelleting of roughages, grains, and complete feeds for cattle indicate potential for the alfalfa dehydration industry, in further expanding usage of their products into ruminant feeding, and in custom pelleting operations during the winter months to provide more efficient utilization of plant facilities and personnel.

PELLETING ROUGHAGE, GRAIN OR COMPLETE RATIONS
FOR BEEF AND DAIRY CATTLE

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After the advantages of pelleting rations for lambs became apparent, it was a logical step for researchers to begin evaluating the possibilities of pelleted rations for beef cattle. This discussion is intended to review the available information on pelleted rations or ration components for beef and dairy cattle, and the recent developments in "wafering" and pelleting roughages.

The advantages of pelleting rations for beef cattle are very much the same as for lambs, and may be listed as follows:

1. Permits bulk handling of feeds with automatic equipment, providing opportunity for labor savings.
2. Permits self-feeding.
3. Reduces chances for mixing and feeding errors by hired labor - every bite is complete ration.
4. Cuts down feed waste, due to wind, sorting by animals, etc.
5. Increases consumption of poor quality roughages.
6. Usually gives improved feed efficiency; rate of gain of cattle may or may not be increased.

PELLETING ROUGHAGE FOR BEEF CATTLE

Several groups of researchers have investigated the effects of feeding pelleted roughage either on a complete ration or as a part of a growing-type ration for beef cattle. These results are summarized in Table I.

In the Illinois tests, the dramatic results obtained from pelleting were due, in part, to the unusually poor gains of steers fed long hay. The alfalfa hay fed in the California experiment was of exceptionally high quality (21% protein). With the exception of the Oklahoma report, these results indicate that the pelleting of roughage may be expected to give increased rate and economy of gain in high-roughage-type rations.

The Illinois (Dixon Springs Experiment Station) research also has demonstrated differences in the nutritive value of various roughages, even when pelleted. Alfalfa hay pellets gave faster and more efficient gains than timothy-alfalfa pellets or Seircea Lcspedeza hay pellets.

PELLETING GRAIN FOR BEEF CATTLE

Reports appearing in the feed trade press indicate that pelleting only the grain in a fattening ration for cattle is of benefit. At the University of Minnesota's Crookston station, steers fed a pelleted grain mixture of 70% barley and 30% oats, gained more and returned \$5.40 more profit per head than steers fed the same ration in non-pelleted form. Oklahoma investigators report that beef calves fed pelleted milo made faster and more efficient gains than heifers fed rolled milo. Feeding pelleted milo increased average daily gain from 2.09 to 2.17 pounds and increased the net return per animal \$11.15.

PELLETING COMPLETE RATIONS FOR BEEF CATTLE

Many groups of researchers are testing complete pelleted rations for fattening cattle, and the current results which are available are summarized in Table II.

In these experiments, pelleting the complete ration has had a variable effect on rate of gain, but has given a marked and consistent improvement in feed efficiency. Carcass data are not yet available from the Purdue experiment. The Standard Research Institute data indicate some improvement in carcass quality as a result of pelleting on the high-roughage ration, but not on the high concentrate ration.

ROUGHAGE TO CONCENTRATION RATIONS FOR FATTENING CATTLE

One of the important findings which has come from the current research with pelleted cattle rations is that high roughage pelleted rations apparently will not produce a satisfactory degree of fatness or market finish. This is a contrast to the results of lamb research, which has shown that a satisfactory market finish can be produced by feeding pelleted rations which are high in roughage.

In the study conducted by the Stanford Research Institute, steers fed rations containing 64% roughage had lower daily gains, dressing percentages, and carcass grades than steers fed rations containing 60% concentrate. In this same series of tests, pelleted rations containing 85 to 95% roughage were even more unsatisfactory for fattening purposes. At the Dixon Springs (Illinois) Experiment Station, a comparison of different concentrate-to-roughage ratios in pelleted rations for fattening steers showed that best results were obtained with a 55% grain level in the absence of stilbestrol implants and with a 65% grain level when steers received stilbestrol implants. A progress report on an experiment at the Ohio Experiment Station indicates that a ration containing 75% ground ear corn and 25% pelleted alfalfa hay is giving better results than rations containing either 50-50%, or 66-7% and 33-3% of corn and alfalfa pellets, respectively.

ECONOMICS OF PELLETED CATTLE FEEDS

The most important consideration in respect to pelleted cattle feeds, from a practical standpoint, is whether or not the reduction in labor costs and the improvement in rate of gain and feed efficiency is great enough to offset the cost of grinding and pelleting the feed.

In the Purdue experiment, the increase in feed efficiency on the pelleted ration lowered feed costs per pound of gain by 1.1 cent, when a \$3.00 per ton pelleting charge was included in calculating feed costs for the pelleted ration.

The most complete data on costs have been assembled in the Stanford Research Institute report, as indicated in Table III.

It can be seen that the labor requirement per lot was reduced 3.5 hours or 8.8% when pelleted feeds were fed. This reduction in labor cost was not great enough to offset the pelleting cost, as indicated by the higher total feed costs in the pellet fed lots when labor and pelleting charges were included. However, because the pelleted feeds gave a reduction of 13.4 to 15.4% in the amount of feed required per unit of gain, the final total feed costs per unit of gain were 10-12% lower with the pelleted feeds as compared with the non-pelleted feeds.

Thus, even though self-feeding pelleted feeds can be expected to reduce labor costs somewhat, it seems apparent that there must also be a substantial reduction in the amount of feed required per unit of gain to give final feed costs equal to or less than those with non-pelleted feed.

On the basis of the research reported to date, the following statements appear to be justified: (This summary should be considered as preliminary in nature, for the results of research now in progress at many institutions may substantially alter present conclusions.)

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1. The pelleting of either poor or good quality roughages for cattle has resulted in increased rate of gain and improved feed efficiency.
2. Quality differences between roughages may still be demonstrated with pelleted roughages.
3. The results of a limited number of experiments indicate that pelleting only the grain portion of the ration for cattle also gives increased rate of gain and improved feed efficiency.
4. The pelleting of complete rations for beef cattle appears to give a consistent and marked improvement in feed efficiency, but has a variable effect on rate of gain.
5. Pelleted high roughage rations apparently will not produce a satisfactory degree of fatness or market finish in beef cattle, as contrasted to sheep: high concentrate levels appear to be necessary in pelleted beef cattle rations to produce most rapid gains and acceptable market finish.
6. Current results indicate that the improvement in feed efficiency due to pelleting and the reduction in labor costs generally give savings in total feed costs sufficient to balance the additional costs of processing and pelleting cattle rations.

WAFERING AND PELLETING OF HAY

At the present time, there is a great deal of interest in the compressing of hay into a wafered or pelleted form as opposed to baling of hay as is now a common practice on farms. As farm units become larger and labor supply and costs become greater problems, farmers are becoming increasingly interested in the use of labor saving processing, handling, and storage methods and equipment. The advantages of compressed or pelleted hay for farmers are quite similar to those listed above for pelleted feeds: permits bulk handling and storage; reduces storage space requirements; reduces labor requirements and costs; reduces feed loss and feed wastage; gives equivalent or better livestock performance; and may lower feed transportation costs.

Considerable publicity has been given to a monstrous field hay pelleting machine built by the Stewart Brothers of Rio Vista, California. The 10-ton machine, which turns out two tons of pellets per hour, costs \$35,000 to build. Although it may be the forerunner of future mobile field-pelleting equipment, this machine is too heavy and expensive for general farm usage. However, this development has emphasized the almost complete lack of portable, economical pelleting equipment, and it is very understanding that processing equipment manufacturers are now hard at work on the problems involved in producing suitable pelleting equipment which may be adapted to farm use.

Another development, of equal interest, and also on the West Coast, has taken place in Oregon.

The Sumner Iron Works, Everett, Washington, working in cooperation with Pendleton (Ore.) Grain Growers, Inc., Beef City, Oregon, have adopted equipment for making fuel briquettes to the production of so-called hay "wafer". The wafers are 1/2" to 1" thick and either 3" or 4" in diameter. The machine operates on a continuous extrusion principle and estimated production rate is 4 to 6 tons per hour. The "wafers" are made from coarsely chopped hay with an initial moisture content of 15 to 20%. It is reported that "wafering" reduces the volume of baled hay approximately three times, chopped hay from 5 to 6 times and loose hay about 10 times.

According to a recent article appearing in Feedstuffs, July 19, 1958, a portable model of this machine, called a "Foragizer" has been made and will be marketed by a West Coast firm. An official of this firm was quoted as stating that the "Hayfers" produced with the machine could be made for a cost not to exceed baling costs and the resulting product can be handled mechanically, eliminating considerable labor costs and substantially reducing storage space requirements. According to the report, the machine can be operated as a portable unit which can be pulled into hay fields or as a stationary unit where the chopped hay is brought to the machine at a central location. Needless to say, this type of equipment has to be classified as experimental at the present time and final evaluation of its worth must be withheld until more is known about its performance under practical conditions.

NUTRITIONAL ASPECTS OF "WAFERS" AND PELLETTED ROUGHAGE FOR DAIRY CATTLE AND OTHER RUMINANTS

A large share of the interest in different types of corn-pressed hay products made from coarsely chopped hay is due to the thinking of many that finely ground roughages tend to depress butterfat production in dairy cows. This is believed to be a result of too rapid passage of the fine roughage particles through the rumen, which presents adequate breakdown of the roughage by rumen micro-organisms or bacteria. While some may consider this type of thinking as only opinion, there is more than a little scientific evidence which indicates finely ground roughages have a depressing effect on butterfat production. There also are indications that fineness of grind of roughage in pellets may be equally important for beef cattle and sheep.

Jones, Magill and Petersen, of Oregon State College have compared baled, wafered, and pelleted second cutting alfalfa hay of good quality, as sources of roughage for lactating dairy cattle. Their results, summarized in Table IV, show that there were no differences between the three physical forms of hay in regard to feed consumption.

actual or 4% fat-corrected milk production, or body weight of cows. However, the feeding of pelleted hay (ground through a 5/16" screen) resulted in a statistically significant reduction in butterfat production, as compared with either baled or wafered hay. There was no difference in butterfat production between cows fed either baled or wafered hay.

Further evidence on this point is found in results from the University of Connecticut, report by Porter et al., and shown in Table V.

In the first experiment, the increased milk flow from cows fed ground or pelleted dehydrated alfalfa tended to wash the reduction in butterfat percentage, since the amounts of fat-corrected milk produced on the dehydrated alfalfa was greater than for the field-cured alfalfa. However, in the second experiment, the chopped dehydrated alfalfa increased milk flow and had no effect on butterfat production, whereas, pelleted dehydrated alfalfa depressed both milk flow and butterfat production. The investigators attributed part of the lowered production or pellets to the fact they were so hard that consumption of pellets was lowered. In a further investigation of differences between pellets fed in the two experiments, the screen analysis of alfalfa in the pellets and the hardness of the pellets were determined. It was found, Tables VI and VII that the alfalfa in the Experiment I pellets had a much coarser grind than in the pellets fed in Experiment II, and consequently, were not nearly so hard.

Thus, this evidence indicates that fineness of grind and hardness of pellets are factors of major importance in pellets intended for dairy cattle feeding.

Another aspect of hay processing has been introduced by University of Maryland researcher, J. C. Shaw, in results presented at the 1958 Distillers Feed Research Conference. His results show that processing of feedstuffs may profoundly affect the intermediary metabolic products produced in the rumen, with a subsequent effect on butterfat production in dairy cows. Some of these results are shown in Table VIII.

The volatile fatty acids produced in the rumen are absorbed directly into the blood stream from the rumen, and serve as sources of energy or as precursors for butterfat production. The production of acetic acid, for example, has been directly associated with butterfat production, so that changes in production of acetic acid in the rumen are reflected in the amount of butterfat produced. Dr. Shaw apparently feels that steaming is the major factor responsible for the changes in rumen fatty acid and butterfat production; however, he has also introduced the factor of feed particle size in the feeding of pellets.

Recent results with lambs at the Colorado Experiment Station provide further evidence of the effects fineness of grind of roughage in pelleted feeds may have on animal performance. These results, Table

IX, show that changing the grind of alfalfa hay in a pelleted lamb feed from 1/16" to 1/4", gave more rapid lamb gains, but did not affect feed efficiency.

The above data are a clear indication that processing very definitely can affect the value of feeds and ingredients for ruminants, and also, that we need to know a great deal more about how to prepare dehydrated alfalfa and complete feed pellets for optimum nutritional value in ruminant feeding.

It would well be that some of the troubles reported with pelleted feeds for ruminants - animals going off feed, chewing fences, eating dirt, outright refusals to eat pellets, digestive disturbances, and lower butterfat production - are direction caused by processing factors. Incorrect processing could also be responsible for the poor performance of dehydrated alfalfa in certain experiment station feeding trials.

It would seem that Experiment Station researchers have a good opportunity to further advance the science of ruminant feeding through careful research into this problem of how best to process roughages and rations for optimum performance in beef cattle, dairy cattle, and sheep.

SUMMARY

1. Although more research is needed, the pelleting of roughages, grains, or complete feeds for ruminants appears to have considerable potential for improving animal performance and permitting greater use of labor-saving methods and equipment by farmers and feeders.
2. Pelleted feeds for ruminants offer possibilities for increased ruminant feed volume for feed manufacturers.
3. Pelleting developments appear to offer considerable potential for alfalfa dehydrators, in expanding the existing market for dehydrated alfalfa products; and in custom grinding and pelleting of roughages, grains, or complete feeds in off production season.
4. More research is needed to determine the best processing methods to use in the production of pelleted dehydrated alfalfa, other roughages and complete feeds.

Table I

COMPARISON OF PELLETED VS. NON-PELLETED HAY FOR STEERS

	% Change Due to Pelleting			
	<u>Avg. Daily Gain</u>	<u>Feed Per Cwt. Gain</u>	<u>Dressing Percent</u>	<u>Carcass Grade</u>
Illinois	+175	-44.9	-	-
Illinois	+883	-84.6	-	-
California	+ 20.5	- 3.5	+1.1	Improved
Oklahoma	0.0*	-	-	-

*Data unavailable; reported that steer calves on 108 day trial made greater gains on chopped, as compared with pelleted roughage.

Table II

COMPARISON OF PELLETED VS. NON-PELLETED COMPLETE
RATIONS FOR BEEF CATTLE

	% Change Due To Pelleting				
	<u>Avg. Daily Gain</u>	<u>Feed Per Cwt. Gain</u>	<u>Feed Cost Per Lb. Gain</u>	<u>Dressing Percent</u>	<u>Carcass Grade</u>
Purdue*	-11.3	-13.7	-1.1¢	--	--
SRI - High Roughage**	+ 6.5(N.S.)	-15.4	-4.23¢	+1.5	Improved
SRI - High Concentrate**	0.0	-13.4	-4.29¢	0.0	No change

*Mimeo AH 228, April 25, 1958. Purdue University Agricultural Experiment Station.

**Stanford Research Institute, Menlo Park, California. Report of experiments conducted at Rancho Quien Sabe, Hollister, California, in 1957.

Table III

COMPARATIVE COSTS OF FEEDING STEERS PELLETTED
OR NON-PELLETED RATIONS

	High <u>Mix</u>	Roughage <u>Pellet</u>	High <u>Mix</u>	Concentrate <u>Pellet</u>
Feed (only) cost per ton	38.55	38.55	41.11	41.04
Labor, hrs. (40 steers, 120 da)*				
Feeding	14.	10.5	14.	10.5
Other (cleaning, branding, weighing, repairs, etc.)	25.5	25.5	25.5	25.5
Labor cost per lot, \$	39.50	36.00	39.50	36.00
Milling cost per ton, \$	6.73	6.73	6.73	6.73
Pelleting cost per ton, \$		2.50		2.50
Total feed cost/ton, \$	45.78	48.44	47.38	50.71
Total feed cost/cwt. gain, \$	32.21	28.70	28.92	26.30

*Labor charged at \$1.00 per hour.

Table IV

EFFECTS OF FEEDING BALED, WAFERED OR PELLETTED
ALFALFA HAY TO DAIRY COWS*

	Form of Alfalfa Hay		
	<u>Baled</u>	<u>Wafered</u>	<u>Pelleted</u>
Feed consumed, lb.	797.2	833.2	811.8
Body Weight, lb.	1195.6	1212.9	1190.6
Butterfat, %	4.13	4.15	4.00
Total milk produced, lb.	899.74	887.06	903.93
4% FCM, lb.	848.83	857.14	853.24

*Reference: Oregon State College Reprint, May, 1958.

Table V

EFFECT OF PHYSICAL FORM OF ALFALFA ON MILK AND BUTTERFAT
PRODUCTION IN DAIRY CATTLE

Form of Alfalfa			
<u>Experiment I</u>	<u>Baled*</u>	<u>Ground**</u>	<u>Pelleted**</u>
Actual Milk, lb.	19,433.	20,884.	21,709.
Fat, lb.	792.	800.	797.
Fat, %	4.07	3.83	3.67
FCM, lb.	19,651.	20,346.	20,641.
<u>Experiment II</u>	<u>Baled*</u>	<u>Chopped**</u>	<u>Pelleted**</u>
Actual Milk, lb.	16,328.	16,866.	16,144.
Fat, lb.	693.	710.	610.
Fat, %	4.24	4.21	3.78
FCM, lb.	16,925.	17,393.	15,702.

*Field-cured.

**Dehydrated.

Reference: J. Dairy Science 36:1140

Table VI

SCREEN ANALYSIS OF ALFALFA IN PELLETS FED TO
DAIRY CATTLE IN TWO EXPERIMENTS

<u>Screen Analysis</u>	<u>Experiment I</u>	<u>Experiment II</u>
% over No. 16	6.7	0
% over No. 20	17.0	0
% over No. 30	18.7	5.7
% over No. 40	16.9	12.1
% over No. 50	14.6	16.3
% thru No. 50	26.8	65.9

Reference: J. Dairy Science 36:1140

Table VII

HARDNESS OF PELLETS FED TO DAIRY
CATTLE IN TWO EXPERIMENTS

	Average Crushing Point of Pellets <u>11 lb. per sq. in.</u>
Experiment I	205
Experiment II	468

Reference: J. Dairy Science 36:1140

Table VIII

EFFECT OF FEED TREATMENT ON PROPORTIONS OF RUMEN VOLATILE
FATTY ACIDS AND BUTTERFAT PRODUCTION

	MOLAR PERCENT VFA			Change in
	<u>Acetate</u>	<u>Propionate</u>	<u>Butyrate</u>	<u>Butterfat %</u>
Alfalfa hay + corn	60.3	21.6	13.7	None
Steamed hay pellets + corn	51.5	33.7	10.8	Marked Decrease

Reference: Proceedings, 1958 Distillers Feed Research Conference,
Vol. 13 pp. 74-79